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THE STRUCTURE AND FUNCTION OF THE DIGESTIVE SYSTEM OF THE MUD SNAIL *NASSARIUS OBSOLETUS* (SAY)[1]

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ABSTRACT

The American Atlantic coast mud snail, *Nassarius obsoletus* (Say) is a member of the typically carnivorous rachiglossan Gastropoda. In nature, however, *N. obsoletus* is a non-selective deposit-feeder subsisting almost entirely on ingested sand and mud. The present study was undertaken to clarify the mechanism of functioning of the digestive system of this animal.

Anatomical and histological studies indicate that *Nassarius obsoletus* has all of the structural modifications associated with assumption of a carnivorous mode of existence. These modifications include: an elongate protrusible proboscis; rachiglossan radular dentition; an elongate, movable siphon and bipectinate osphradium; well-developed valve of Leiblein, gland of Leiblein, and salivary glands; a simplified stomach possessing a very reduced gastric shield; no efficient ciliary sorting areas; and well-developed muscular layers surrounding the alimentary canal. In contrast to these clearly carnivorous characteristics, *N. obsoletus* possesses a mucoprotein crystalline style within its stomach – a feature associated with structural adaptation for handling a herbivorous diet. Histochemical studies indicate that the midgut gland contains enzymes capable of splitting esters and glucuronides and thus for metabolizing some of the principal constituents of algae. Feeding experiments using finely divided particulate material and histochemical localization of phosphatase activity both indicate that phagocytosis and intracellular digestion do not occur. *In vitro* enzyme analyses of tissue homogenates of the various digestive organs reveal the presence of esterase, lipase, α-amylase, protease, and several disaccharases. Analyses of stomach fluid and crystalline styles similarly reveal the presence of the hydrolytic enzymes extracellularly within the lumen of the stomach. A review of the feeding habits and behavior is presented along with physiological evidence that the crystalline style aids in the digestive process and is therefore truly functional, rather than being merely a remnant of the mucous fecal string.

It is concluded from the data presented that *Nassarius obsoletus*, although structurally possessing all the features of a typical carnivorous rachiglossan nevertheless is able to subsist almost entirely on a diet of algal detritus; that it possesses the hydrolytic enzymes necessary to breakdown the principal constituents of algae; that the initial breakdown occurs extracellularly; that phagocytosis and intracellular digestion do not occur; and that absorption of soluble digestion products occurs most probably in the midgut gland or epithelium lining the stomach-intestine.

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CONTENTS

I. INTRODUCTION

The gastropod genus *Nassarius* (Prosobranchia, Neogastropoda) is world-wide in distribution. A representative of this genus, *Nassarius* (*Ilyanassa*) *obsoletus* (Say), is one of the most abundant animals of the intertidal mud flats along the Atlantic Coast of North America.

Although much of the basic biology of *Nassarius obsoletus* has not been studied in detail, the animal has, nevertheless, been the subject of many experimental and descriptive studies. These have been in the areas of: experimental embryology (Crampton, 1896; Morgan, 1933; Dan & Dan, 1942; Clement, 1952, 1956, 1960 and 1962; Clement & Lehmann, 1956a and 1956b; Berg & Kato, 1959; Cather, 1959, 1963 and 1967; Collier, 1960 and 1961; Clement & Tyler, 1967); larval development (Scheltema, 1956, 1961, 1962a, 1962b and 1965; Paulson & Scheltema, 1967); behavior and physiological ecology (Dimon, 1905; Batchelder, 1915; Stephens, *et al*, 1953; Jenner & Chamberlain, 1955; Jenner, 1956a, 1956b, 1957 and 1958; Baylor, 1958; Brown, *et al*, 1959 and 1960; Scheltema, 1964; Nagabhushanam & Sarojini, 1965; Carr, 1967a and 1967b); and parasitology (Martin, 1938 and 1939; Stunkard, 1938a, 1938b and 1961; Rankin, 1940; Stunkard & Hinchliffe, 1952; Sindermann, 1960; Printz, 1962).

The results of several of these studies point to the fact that the feeding habits and digestive system of *Nassarius obsoletus* show features which are quite unusual for a member of the Neogastropoda. Although neogastropods are regarded as primarily carnivorous (Fretter & Graham, 1962; Hyman, 1967) and all other species of *Nassarius* which have been studied are classified as carnivores (Yonge, 1954; Martoja 1964), *Nassarius obsoletus* has been reported (Jenner, 1956b) to possess a crystalline style, a structure considered to be a definitive characteristic of purely herbivorous molluscs (Yonge, 1930). Scheltema (1956 and 1961) has presented evidence strongly suggesting that the feeding habits and perhaps even nutritional requirements of adult *N. obsoletus* are of prime importance in determining the time and place of settling and metamorphosis of their planktonic veliger larvae.

The present study was undertaken to elucidate the mechanism by which the digestive system of this animal functions. The results are presented in four parts: tissue and organ structure; enzyme histochemistry; *in vitro* enzyme analyses; and digestive physiology and behavior. A brief evaluation is presented at the end of each part, dealing with that section. The general discussion at the end attempts a synthesis of the data into a coherent picture of structure and function.

II. ANATOMY AND HISTOLOGY

1. Materials and methods

All descriptions are based on fresh and preserved specimens collected from the vicinities of Woods Hole and Barnstable Harbor, Massachusetts. In some cases,

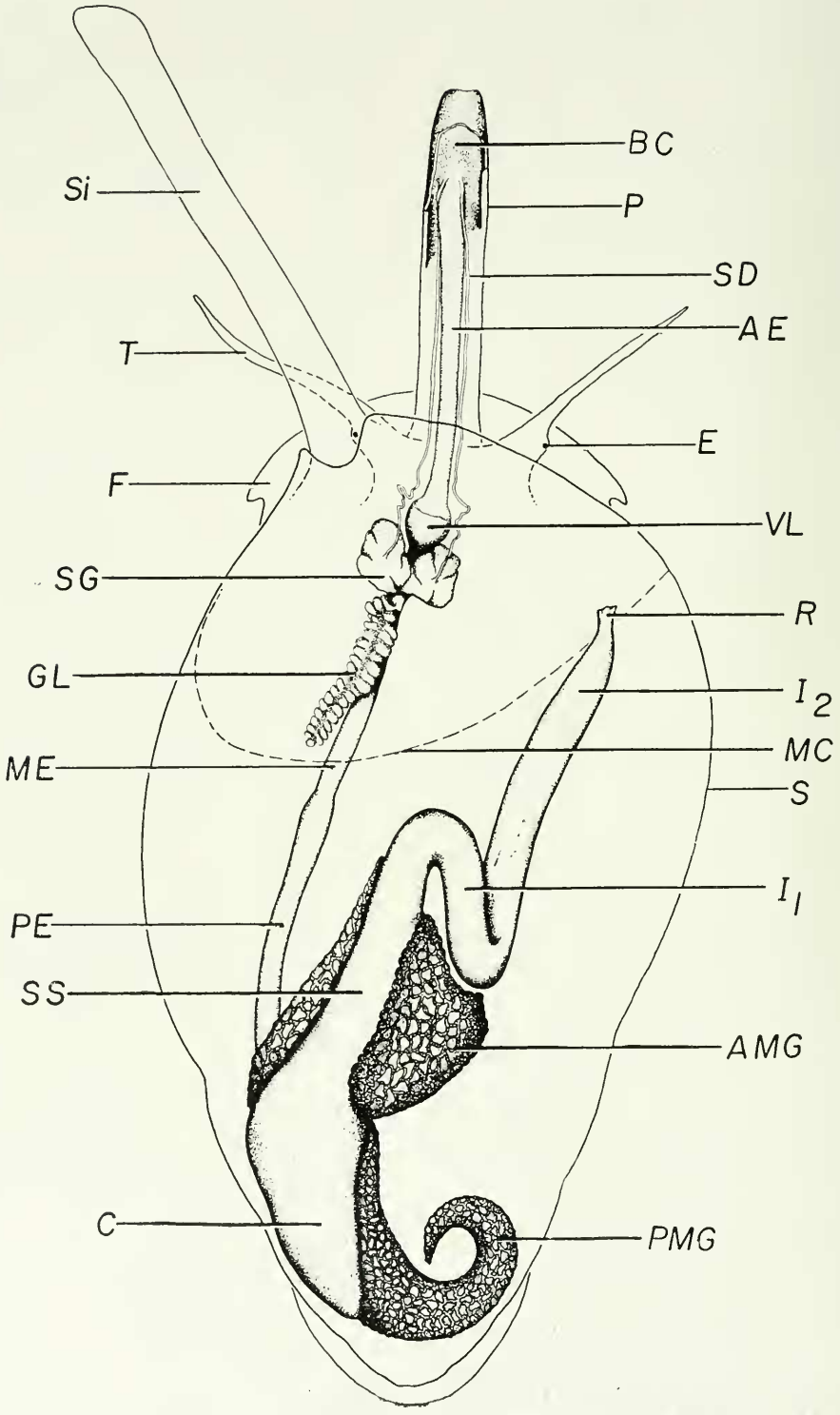
animals were maintained at the University of Michigan in sea-water aquaria on a diet of frozen shrimp prior to fixation or examination. Dissections were carried out on living animals and also on those previously hardened in ten percent formalin.

For most general histological work Carnoy's, Bouin's, Zenker's, Helly's and Atkins' fixatives were used, with the first two giving the best nuclear detail while the last three yielded the clearest overall histological results. Materials so fixed were paraffin embedded, sectioned at 4–10 microns, and stained with Heidenhain's iron hematoxylin, Weigert's iron hematoxylin with Orange G, Heidenhain's azan, Mayer's mucicarmine, and Bismarck brown with methyl green. In addition, some salivary gland and midgut gland material was fixed in acrolein or glutaraldehyde and embedded in Epon according to the method of Luft (1961). This Epon-embedded material was then sectioned at one-half to 2 microns on a Porter-Blum ultramicrotome with a glass knife and subsequently stained with Azure B bromide at pH 8·0, Weigert's iron hematoxylin with Alcian blue, or Toluidine blue in 2·5% sodium carbonate.

Special techniques employed for the detection of tissue components and for the characterization of mucins included: the coupled tetrazonium reaction for proteins, using Fast blue B salt as coupler (Burstone, 1955); the DMAB-Nitrite method for tryptophan on formalin-fixed tissues (Adams, 1957); the Periodic acid —Schiff (PAS) technique for vicinal hydroxyl groups using Lillie's "cold Schiff" reagent (Lillie, 1965); the PAS reaction preceded by digestion in 1/1000 malt diastase for one hour; the standard toluidine blue method for metachromatic substances (Pearse, 1960); toluidine blue preceded by digestion for up to 24 hours in bovine testicular hyaluronidase; the Alcian blue method for acid mucopolysaccharides carried out at a pH below 2 (Steedman, 1950; Mowry, 1963); the combined Alcian blue—PAS technique according to Mowry (1963); the dialysed iron method for acid mucopolysaccharides (Hale, 1946; Mowry, 1963); and the methylene blue extinction technique according to Dempsey & Singer (1946). In addition, formalin-fixed tissue was stained for calcium with Nuclear fast red according to the method of McGee-Russell (1958) and endogenous iron was detected by the Prussian blue reaction on formalin-fixed, paraffin embedded material.

2. Organ and tissue structure

The digestive tract of *Nassarius obsoletus* (Fig. 1) is similar to those of the European species, *N. reticulatus*, and *N. incrassatus*, figured by Fretter & Graham (1962) and Martoja (1964). At the apex of the long pleurembolic proboscis lies the buccal cavity. A pair of salivary ducts open into the dorsal posterior aspect of this cavity just anterior to the level where the esophagus originates. The long esophagus can be divided, following Graham's (1939 and 1941) terminology, into the following regions: the anterior esophagus, extending dorsal to the radular mass from the buccal cavity to the valve of Leiblein; the midesophagus, commencing with the valve of Leiblein, proceeding through the nerve ring—salivary gland complex, and terminating posterior to the gland of Leiblein and its opening; and the postesophagus, continuing posteriorly and ending at its stomach opening which lies at a level between the posterior caecum and the anteriorly-directed style sac. The posterior and anterior midgut glands almost completely envelop the caecum and style sac, respectively. Anteriorly from the style sac, the intestine makes a sharp S-curve at the level of the heart and kidney and then arches forward dorsally within the mantle. A rectal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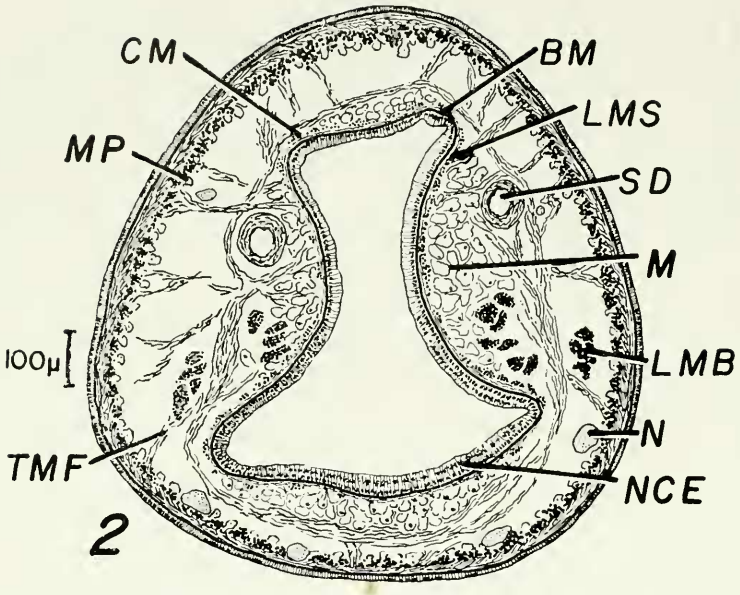
papilla protrudes from the roof of the mantle cavity on the right side.

Buccal region: The ventrally-directed triangular mouth delimits the anterior border of the buccal cavity. The cavity itself (Figs. 2 and 3) is roughly triangular in cross section. As it extends posteriorly, a horizontal partition divides it into a dorsal chamber which soon leads into the anterior esophagus and a ventral chamber (Fig. 3, C-E) which encloses the odontophore and radular apparatus (not shown).

The buccal cavity is lined with a smooth layer of simple columnar epithelium (Fig. 2, NCE) the cells of which have basally located oval nuclei. The cells

FIG. 1. The digestive system of *Nassarius obsoletus*, viewed dorsally. The apex of the visceral mass has been uncoiled slightly for illustrative purposes. For interpretation of the lettering on this and following figures, see Key to Abbreviations below:

AE	Anterior esophagus
AMG	Anterior midgut gland
BC	Buccal cavity
BM	Basement membrane
BV	Blood vessel
C	Caecum
CC_1, CC_2	Columnar cells, types 1 and 2, of midgut gland
CCC	Ciliated columnar cell
CE	Ciliated epithelium
CF	Ciliated folds
Cil	Cilia
CM	Circular muscle
CP	Conical papilla
CS	Outline of crystalline style
CT	Connective tissue
Cut	Cuticle
DC	Dorsal chamber
DF	Remnants of primitive dorsal fold
E	Eye
EMC	Epithelium of mantle cavity
F	Foot
G	Granules in lumen of salivary duct
GC	Epithelial granule cell
GC_1, GC_2	Granule cells, types 1 and 2, of salivary gland
GCF	Granule cell fragments
GL	Gland of Leiblein
GS	Gastric shield
H	Haemocyte
I_1, I_2	Regions 1 and 2 of intestine
IG	Intestinal groove
L	Lumen of gland or duct
LM	Longitudinal muscle
LMB	Longitudinal muscle bundles
LMS	Longitudinal muscle sheath
LS	Lateral sulcus
M	Mucous cells
MaT	Major typhlosole
MC	Mantle cavity outline
ME	Midesophagus
MG	Midgut glands
MGC	Mucous goblet cell
MGD	Duct of midgut gland
MiT	Minor typhlosole
Mo	Mouth
MO	Midgut gland openings
MP	Mucous cells of proboscis outer epithelium
MS	Mucus string adherent to style
Mu	Mucin in salivary duct
N	Nerve
NCE	Non-ciliated columnar epithelium
Nu	Nucleus
OPE	Opening from posterior esophagus
P	Proboscis
PE	Posterior esophagus
PG	Pigment Granules
PMG	Posterior midgut gland
PsE	Pseudostratified epithelium
R	Rectum
RCC	Ring of ciliated cells
RI	Refractile inclusions
RP	Reaction product
S	Shell outline
SD	Salivary duct
SG	Salivary gland
Si	Siphon
Sp	Septum
SR	Stomach region
SS	Style sac
SSA	Saddle-shaped area of stomach
StH	Style head
StS	Style shaft
T	Tentacle
TC	Triangular cell
TMC	Expanded tip of mucous cell
TME	Thickened wall of midesophagus
TMF	Transverse muscle fibers
Ty	Typhlosole
VC	Ventral chamber
VG	Ventral groove
VL	Valve of Leiblein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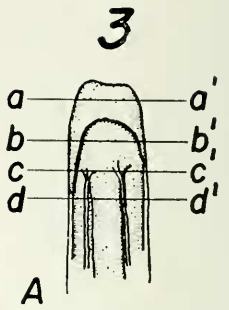
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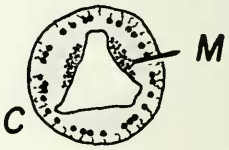
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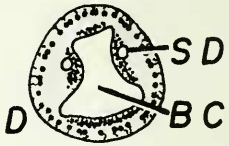
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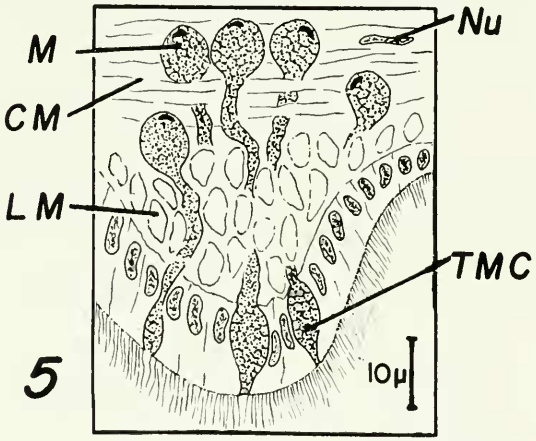
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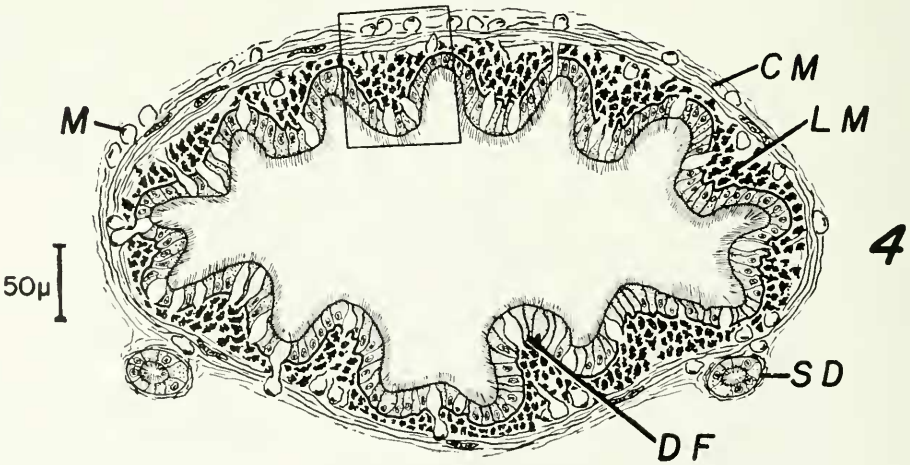
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are non-ciliated but often contain very fine black pigment granules scattered in the apical 1/3 of the cells. Underlying the epithelial layer is a prominent basement membrane which stains very strongly with Schiff's reagent or with aniline in preparations stained with Heidenhain's Azan. Immediately beneath this basement membrane lies a thin irregular layer of longitudinally directed muscle fibers interspersed with a rather loose connective tissue. Along the walls of the buccal cavity and beneath the muscle layer lie 4 large concentrations of mucous gland cells (Fig. 2. M. and Fig. 29). These large gland cells have dense basally located semilunar nuclei and are of the unicellular type, each communicating with the lumen of the buccal cavity by a conspicuous neck which can be traced through the muscle layer and basement membrane and emerging between the cells of the lining epithelium. The gland cells contain a PAS-positive acid mucopolysaccharide as shown by the PAS reaction, toluidine blue metachromasia, the Hale and Alcian blue techniques, and by a methylene blue extinction point of less than 2.

Underlying these elements is a large haemocoelic cavity traversed by three sets of muscles directed as follows: a thin continuous band of circular muscles (Fig. 2. CM) loosely enveloping the buccal complex; 4 sets of longitudinal muscle bundles (LMB) lying in the ventral half of the proboscis; and an irregular complement of transverse muscle fibers (TMF) inserting in the connective tissue underlying the proboscis epithelium. As stetad above, the salivary ducts empty dorsolaterally into the rear of the buccal cavity. In cross sections taken at the posterior levels of the cavity, the terminal portions of the salivary ducts can be seen lateral to the mucous gland cells and just beneath the circular muscle layer (SD). The salivary ducts at this level are composed of a very thin endothelium surrounded by a relatively thick coat of circularly directed smooth muscle.

Anterior esophagus: The anterior esophagus, like the rest of the esophagus, is characterized by the presence of conspicuous longitudinally folded walls (Fig. 4). These folds, in the anterior esophagus, are of similar size with the exception of the 2 folds which occupy the mid-ventral position (DF). These two folds are somewhat larger than the rest and the furrow between them is noticably larger. As Graham has shown (1939), these folds are the remnants of the primitive dorsal folds which have migrated ventrally and have thus expanded the originally dorsal food channel to include virtually the entire area of the esophagus with the exception of the present mid-ventral furrow.

The epithelium lining the anterior esophagus is of the simple columnar type having oval subcentral nuclei (Fig. 5). These epithelial cells, in contrast to those of the buccal cavity, possess long cilia. These ciliated cells are strongly acidophilic at their bases, but exhibit increasing bosophilia at their apices. A prominent basement membrane underlies the epithe-

FIG. 2. Cross-section of the proboscis at the posterior end of the buccal cavity. Heidenhain's Azan.

FIG. 3. Relationship of buccal cavity to proboscis, diagrammatic. **A.** Anterior of proboscis, viewed dorsally, epidermis partially cut away. **B.-E.** Sections through levels a-a' to d-d', respectively.

FIG. 4. Cross-section of anterior esophagus in the region of the middle of the proboscis. Heidenhain's Azan.

FIG. 5. Detail of wall of anterior esophagus, in cross-section. Weigert's iron haematoxylin-Alcian blue.

lial layer. Immediately below this membrane is located a heavy continuous layer of longitudinally directed muscle bundles (Fig. 4, LM) interlaced at irregular intervals with connective tissue. A continuous circular layer of muscle (CM) surrounds the longitudinal muscle fibers.

A distinctive feature of the anterior esophagus is the presence of mucous gland cells lying beneath the longitudinal muscle layer (Figs. 4 and 5, M). Most of these mucous cells lie outside the circular muscle coat, but a few of the cell bodies may be found between the 2 muscle layers. These submucosal gland cells are similar in structure to those underlying the buccal cavity, having similar dimensions and dense semilunar nuclei disposed towards the base of the cells. The mucin within these cells, a PAS-positive acid mucopolysaccharide, is histochemically identical to that of the buccal cavity gland cells (see Table 1 for a comparison of staining properties). The necks of the mucous cells pass through the muscle layers and basement membrane and often dilate at the level of the epithelium to become two to three times as wide as the adjacent ciliated columnar cells (Fig. 5). There are no goblet mucous cells among the ciliated columnar cells lining the lumen of the anterior esophagus.

The salivary ducts accompany the anterior esophagus along the ventrolateral margins, being loosely attached to the circular muscle layer by strands of connective tissue (Fig. 4, SD). The ducts at this level, in contrast to their appearance in the region of the buccal cavity, are composed of a ciliated cuboidal epithelium a single layer thick surrounded by a very thin coat of connective tissue. The lightly-staining nuclei are round and located in the center of the cells. The cytoplasm is uniformly acidophilic with no trace of basophilia. In some preparations the salivary ducts at the level of the anterior esophagus contain granules which stain intensely with acid dyes, Heidenhain's hematoxylin, and several histochemical reagents (Fig. 16 G). Granules of the same size with identical staining characteristics have been observed in the salivary glands and will be more completely described below.

Valve of Leiblein: The anterior esophagus terminates posteriorly near the base of the proboscis at a pear-shaped organ (Figs. 1, 6, and 7) known as the valve of Leiblein (Fretter & Graham, 1962). This organ consists of a posteriorly directed cone-shaped protuberance (Fig. 6, CP) that is enclosed in a chamber formed by the expanded walls of the anterior portion of the midesophagus (Figs. 6 and 7) The inner surface of the valve of Leiblein shows longitudinal folds similar to those of the anterior esophagus, with the exception that no trace of the primitive dorsal folds or midventral furrow can be found.

Histologically, the inner cone-shaped papilla is lined with a continuation of the ciliated simple columnar epithelium found in the anterior esophagus. There are no muscle layers directly underlying this epithelium. Confluent with this papilla lies a ring of tall ciliated columnar epithelial cells so disposed as to give the appearance of a triangle in longitudinal section (Fig. 7 and 15, RCC). These cells have lightly-staining oval nuclei located centrally. The cytoplasmic staining properties of these cells are distinctive. The usual acid and basic counterstains fail entirely to color the cytoplasm, and the PAS reaction is also negative. In contrast, the cells exhibit strong metachromasia with toluidine blue, are colored by the dialysed iron method for acid mucopolysaccharides, and are heavily colored, metachromatically, with methylene blue below pH 2. The Alcian blue method for acid mucopolysaccharides is completely ineffective in staining the cells, however.

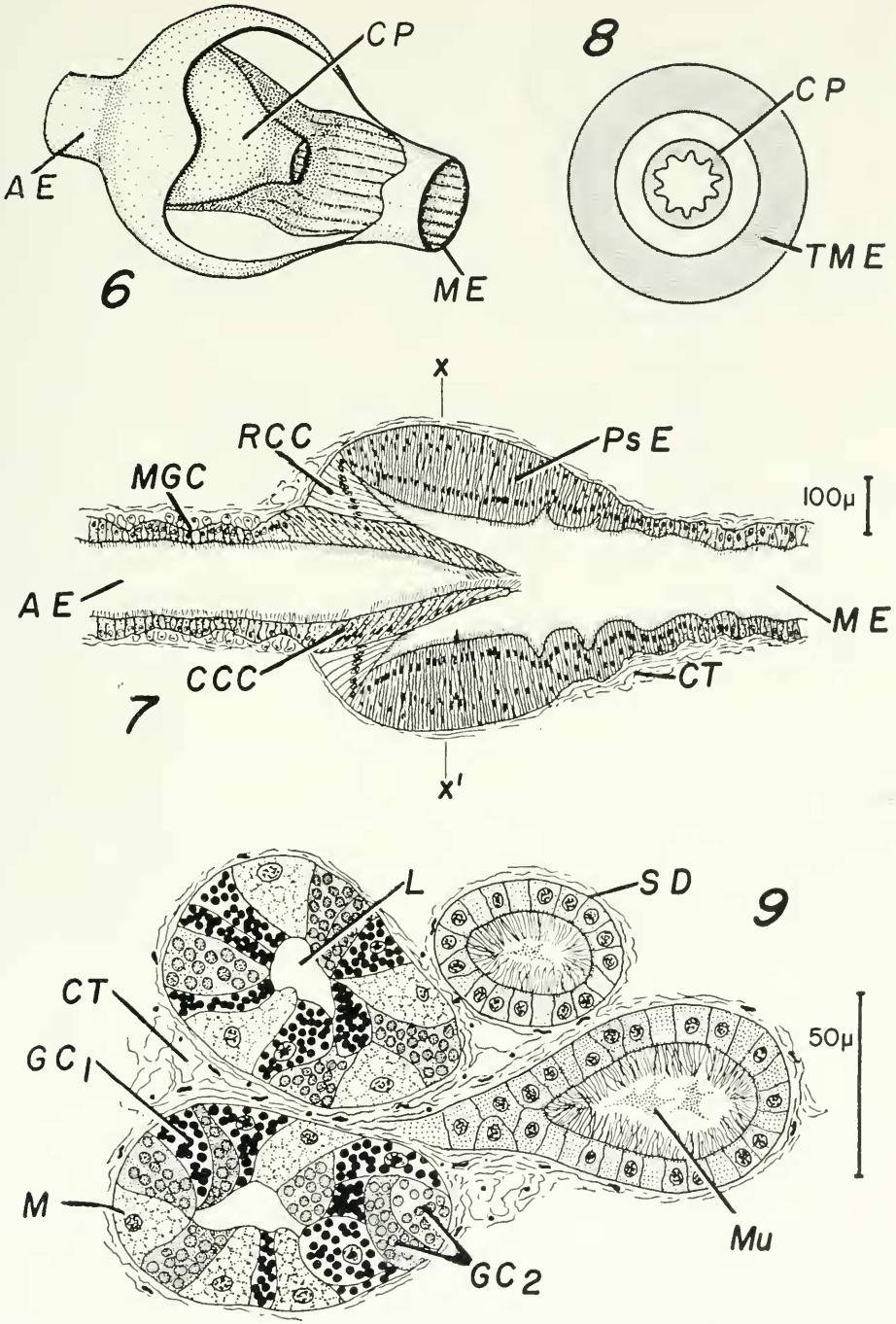
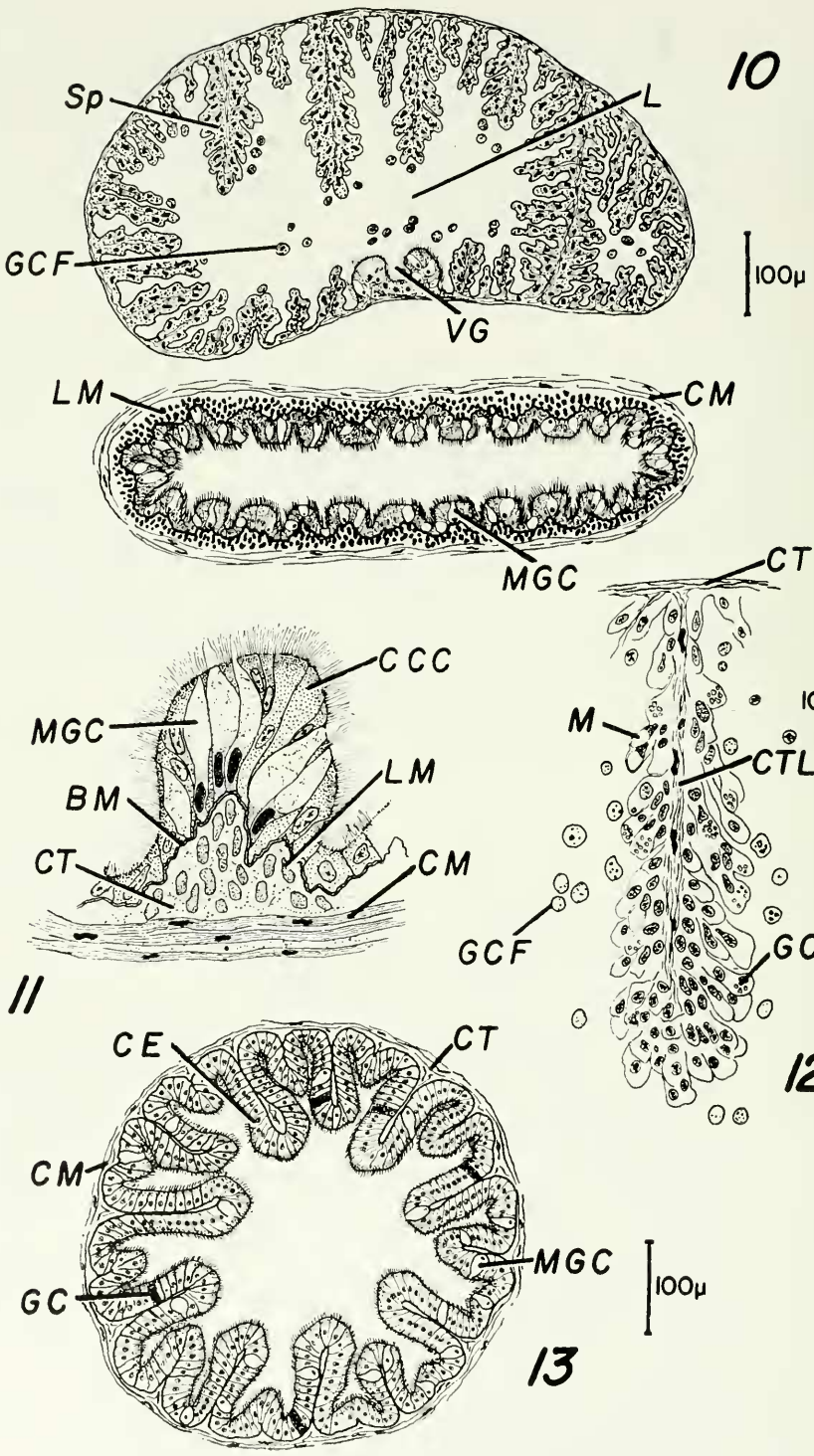


FIG. 6. Stereogram cut-away view of the valve of Leiblein.

FIG. 7. Sagittal section of valve of Leiblein. Heidenhain's Azan.

FIG. 8. Diagrammatic cross-section through valve of Leiblein at level x-x' of figure 7.

FIG. 9. Cross-section through portion of the salivary gland tissue showing ducts and secretory ductules. Heidenhain's haematoxylin-Alcian blue.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The adjacent thickened part of the valve of Leiblein is composed of a pseudostratified columnar epithelium which bears cilia at its lumenal border (Fig. 7). The cells making up this part are of 2 morphological types. The first type extends from basement membrane to lumen, bears cilia, and has dense small nuclei which are very uniformly located 1/5 of the distance from the apical tip. The other type extends from the basement membrane to approximately 2/3 the height of the tissue layer but does not reach the lumen. The nuclei of the cells are scattered in the basal 1/3 of the cytoplasm. The cytoplasmic staining properties of these cells are identical, but, like those described above, differ from the typical pattern. These cells orthochromatically bind methylene blue below pH 2, and are strongly stained by Alcian blue and the dialysed iron reagent, indicative of acid mucopolysaccharides. Contrary to the above, however, these cells remain unstained after treatment with toluidine blue. The pseudostratified layer gradually diminishes in height and merges into the simple columnar epithelium lining the midesophagus.

The outer surface of the valve of Leiblein is covered with a thin coat of connective tissue. There are only a few muscle fibers found in the connective tissue sheath and none arranged in an orderly enough fashion to be termed a true muscle layer.

Midesophagus: The midesophagus continues posteriorly from the valve of Leiblein and passes through the ring of tissue formed by the ganglionic mass and salivary glands. About half way along its length, the midesophagus receives along its mid-dorsal surface the duct from the gland of Leiblein and then continues rearward to the level to the columellar muscle where an externally visible expansion in tube diameter marks the beginning of the postesophagus (Fig. 1). The wall of the midesophagus shows an increase in the number of folds over that of the anterior esophagus, but there is no trace of either dorsal folds or a specialised channel leading into the gland of Leiblein (Fig. 10).

The epithelium lining the midesophagus is of a simple columnar type consisting of three distinct cell types. The most prevalent are ciliated columnar cells with subcentral, oval nuclei (Fig. 11, CCC). These cells are similar to those found in the anterior esophagus and, like them, show an acidophilic character at their bases yielding to basophilia at their apices. These cells make up about 85% of the cell population. The next most numerous type are mucous cells. These cells have the typical goblet shape, being narrow basally and expanding distally (MGC). The nuclei of these cells are dense and elongate and are located basally. The cytoplasm immediately surrounding the nuclei is acidophilic while the mucin at the expanded tip of the cells is a PAS-positive acid mucopolysaccharide (for histochemical characterization, see Table 1). The cells present in the fewest numbers (ca. 1%) are similar in size and shape to the simple columnar cells but differ from them in possessing no cilia and in containing scattered

FIG. 10. Cross-section through the gland of Leiblein (above) and midesophagus (below). Heidenhain's Azan.

FIG. 11. Detail of wall of midesophagus, in cross-section. Heidenhain's Azan.

FIG. 12. Detail of septum of gland of Leiblein, in cross-section. Heidenhain's haematoxylin.

FIG. 13. Cross-section of post-esophagus. Heidenhain's Azan.

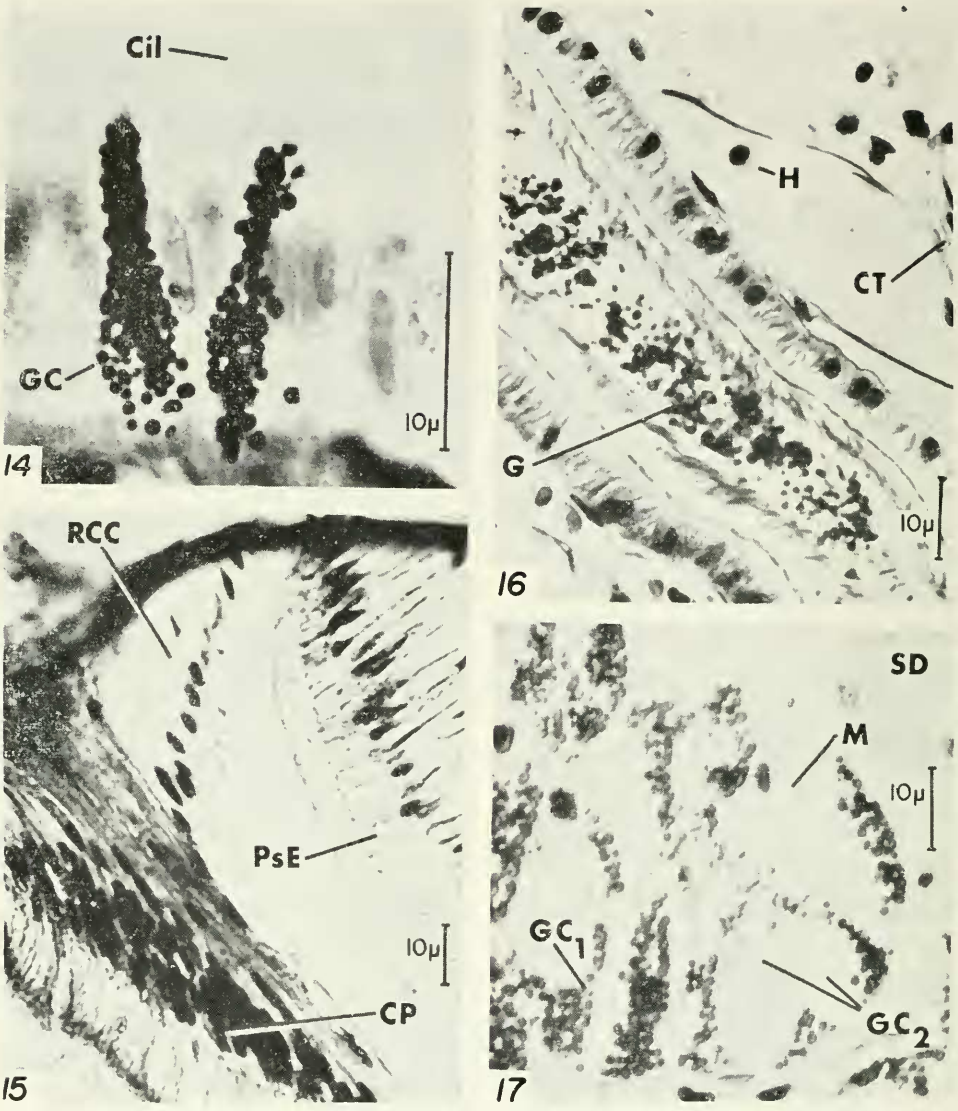


FIG. 14. Epithelium lining the midesophagus, showing cells containing mucoprotein granules. Heidenhain's haematoxylin.

FIG. 15. Sagittal section through the valve of Leiblein in the area of the ring of ciliated cells. Weigert's iron haematoxylin-Orange G.

FIG. 16. Salivary duct at the level of the valve of Leiblein. Heidenhain's haematoxylin.

FIG. 17. Secretory ductule of the salivary gland. Heidenhain's haematoxylin.

throughout their cytoplasm small granules (Fig. 14, GC) which stain intensely with acid dyes and Heidenhain's hematoxylin. The glycoprotein nature of these granules is shown, histochemically, by the facts that they are PAS-positive, are strongly stained by the coupled tetrazonium reaction for proteins, and that they have a

methylene blue extinction point in excess of 6.

The heavy inner layer of longitudinal muscle fibers surrounded by an outer circular muscle coat (Figs. 10 and 11) is identical with that of the anterior esophagus. There are, however, no submucosal gland cells present in the midesophagus.

Salivary glands: The pair of salivary glands which superficially appear to be a single entity form a horse-shoe-shaped structure partially surrounding the dorsal and lateral aspects of the midesophagus, posterior to the valve of Leiblein and in contact with the anterior surface of the ganglionic ring (Fig. 1, SG). After careful removal of the connective tissue surrounding the glands, however, one can observe that the white lobular tissue is divided into two discrete organs, each with its own duct leading from the approximate center of its anterior surface, past the valve of Leiblein, and into the proboscis lateral to the anterior esophagus.

The glands themselves are of the acinar type with small ductules ramifying through the mass of tissue. Lining the ductules is a layer of nonciliated columnar epithelium usually only a single cell in depth and composed of 3 morphologically distinct types of cells present in approximately equal numbers (Fig. 9). The first type of cell (Fig. 9, M) generally has a triangular shape with the base being equal to the height of the cell. These cells have round, lightly-staining nuclei located subcentrally and are filled with a mucin as indicated by the avidity with which they take up mucicarmine and Bismark brown. Histochemical procedures further indicate that this mucin is a PAS-negative acid mucopolysaccharide (see Table 1). The 2nd type of cell (Figs. 9 and 17 GC_1) is usually of a more typically columnar shape, although the apical end is often expanded. These cells have a round central nucleus with a single prominent nucleolus and are filled with large granules which stain very intensely with azocarmine B and Heidenhain's hematoxylin. These granules are also very intensely stained by the coupled tetrazonium procedure for proteins, the DMAB-nitrite reaction for tryptophan, and the PAS technique, all indicative of a glycoprotein composition. These granules are identical in size and staining characteristics with the granules found in the salivary ducts. The cytoplasmic ground substance of these cells fails to take up either acid or basic dyes. The third type of cell (Figs. 9 and 17, GC_2) is structurally similar to the preceding but differs markedly in its staining properties. This cell type shows more variation in the intensity with which the structures are stained than the previous type, but, in general, the granules show less to much less affinity towards hematoxylin and azocarmine while the ground cytoplasm exhibits strong to weak affinities for these dyes. The same variation in intensity is to be seen with the histochemical stains, the spherules being especially conspicuous in never being colored as intensely as those of type 2 cells. Within this variation, a consistent pattern can be observed with regard to the relative staining intensity of ground substance and granules. In the majority of cases the 2 show an equal affinity for the dyes, while the remaining cells of this type can be arranged in a scale of decreasingly stained cytoplasm with a corresponding increase in the intensity with which the granules are colored (Fig. 9, GC_2). Very probably the variation observed in these cell types is correlated with a differentiation of the granules culminating in the definitive cell type described as type 2.

Ciliated salivary ducts with a structure identical to that described above are found throughout the salivary glands. These ducts often contain the intensely staining granules and/or an amorphous material with acid mucopolysaccharide

staining characteristics (Mu). The glandular tissue of the salivary glands is held together by a thin matrix of connective tissue. There are very few smooth muscle fibers present.

Gland of Leiblein: The gland of Leiblein is a single, elongate organ which lies immediately behind the salivary gland /ganglion complex on the dorsal surface of the midesophagus (Fig. 1, GL). This tan to brown organ is connected at its anterior end by a short duct to the middorsal surface of the midesophagus. The gland tapers gradulally at its free posteror end and slight lateral indentations are observable along its length. Internally the gland is of the monopodial branching type and septa just inward laterally at placieble corresponding to the externally visible indentations. The spacious lumen Fig. 10) is partially divided by these septa while a conspicuous midventral groove (Fig. 10, VG) bounded by a pair of folds runs down the axis of the gland and into the duct, eventually merging with one of the grooves of the dorsal wall of the midesophagus.

Histologically the septa are made of thin connective tissue lamellae covered with a pseudostratified columnar epithelium so arranged that in cross section they have a feather-like appearance (Fig. 10, Sp). Two types of calls can be seen lining the septal walls: granular and mucous. The granular cells are of the columnar type with basal oval nuclei (Fig. 12, GC). The cytoplasm of these cells is acidophilic at the base but has little affinity for acid dyes at the cell apex. The colorless tips of the cells are usually expanded where they reach the lumen and are filled with granules and vacuolus of various sizes and shapes. Next to the septa and indeed throughout the lumen of the gland can be found what are presumably nipped-off tips of these cells (Figs. 10 and 12, GCF) containing granules resulting from an apocrine type of secretion of the granular septal cells. Histochemical procedures indicate that the granular contents of both the free cell fragments and the tips of the septal cells are principally mucoprotein (see Table 1). The mucous cells (Fig. 12, M) are of the typical goblet type, containing a PAS-positive acid mucopolysaccharide, and are scattered sparsely throughout the septal walls.

The ventral folds are composed of a ciliated simple columnar epithelium which is reduced to a ciliated cuboidal epithelium in the furrow of the ventral groove (Fig. 10, VG). Interspersed with the columnar cells are typical mucous goblet cells containing a PAS-positive acid mucopolysaccharide. Covering the entire gland of Leiblein is a thin sheet of connective tissue which is confluent with the lamellar cores of the septa. Little if any muscle is present.

Postesophagus: The beginning of the postesophagus is marked by an expansion in the diameter of the tube. Accompanying this, internally, the folds have made a marked increase in depth, although the number of folds remains approximately the same (Fig. 13).

Histologically the epithelium lining the lumen is identical to that of the midesophagus. Here, again, ciliated columnar cells predominate. Also present in small numbers are goblet cells containing PAS-positive acid mucopolysaccharide and non-ciliated columnar cells containing glycoprotein granules. A conspicuous difference is found, however, in the subepithelial structure. In contrast to the heavy inner longitudinal and outer circular muscle layers found encasing the midesophagus, only an extremely thin layer of circular muscle fibers is found surrounding the postesophagus (Fig. 13, CM). In addition, only a small amount of connective tissue is to be found beneath the basement membrane underlying the epithelium and the muscle layer.

Stomach: Viewed from the dorsal aspect, the stomach has the shape of an elongate tubular sac which assumes the form of a semicircle as it spirals apically with the rest of the visceral mass (Fig. 1). The stomach is widest in girth at its middle, where the postesophagus enters ventrally, and then gradually tapers to a bluntly rounded tip at the apical end. The midgut glands closely envelop the stomach except at the left dorso-lateral surface (Fig. 1, AMG and PMG). The expanded midpart of the stomach approximately corresponds with its internal division into caecum (posterior) and style sac (anterior). The caecum (Figs. 1, and 18, C) is a cone-shaped bag at the anterior edge of which the postesophagus empties midventrally. The walls of the caecum are thrown into numerous low folds running longitudinally. Towards the left side, just anterior to the opening of the esophagus, lies an area of ciliated folds converging on a smooth saddle-shaped prominence (Fig. 18, SSA). To the right of the esophageal opening lies a low, longitudinally directed ridge, at either end of which are located the openings to the midgut glands (MO). Immediately to the right of the low ridge lies a large area of smooth epithelium along whose most median edge there often lies a delicate sheet of transparent cuticle, the gastric shield (GS). This last-named structure, when present, can easily be lifted in its entirety from the underlying epithelium and viewed separately. The gastric shield of *N. obsoletus* is unusual in that it is not found in all specimens. Those animals recently collected from the field almost always have the shield present, but they are absent from the majority of animals maintained for any extensive length of time in the laboratory on a diet of frozen shrimp.

Just anterior to the above-mentioned areas lies a deep transverse sulcus (LS) which is in open communication with the midventral longitudinally directed intestinal groove (IG). Bounding this groove on either side lie 2 large ciliated ridges, the major and minor typhlosoles (MaT and MiT). The minor typhlosole forms the left border of the intestinal groove and terminates anteriorly at the end of the style sac where the first region of the intestine makes an abrupt curve to the right. Along the right side of the intestinal groove runs the major typhlosole. It is somewhat wider than the minor typhlosole and instead of terminating at the end of the style sac it continues into the intestine, accompanying it through the sigmoid curve before gently blending into the intestinal wall.

In most animals recently collected from the field, the style sac will be filled by a gelatinous rod whose core may be filled to a varying degree with sand particles and algal detritus. This rod is the crystalline style (CS). The style of *Nassarius* (Fig. 24), when present, usually extends the entire length of the style sac. Anteriorly it tapers to a fine point, while the other end, which extends rearward as far as the gastric shield, is blunt or mushroom-shaped and often has debris or a ropy mucous string adherent to it. Like the gastric shield, the crystalline style is almost always present in those animals examined in the field, but it is absent from the majority of animals maintained for any extensive length of time in the laboratory on a diet of frozen shrimp. Laboratory animals which are not fed shrimp but have access to algal scum almost always possess both shield and style.

Histologically, the lining of the caecum contains mucous goblet cells (Fig. 21, MGC) and granule-filled nonciliated columnar cells (NCE) structurally and histochemically identical with their counterparts in the mid- and postesophagus (see also Table 1). The predominent cell type is of the simple columnar variety,

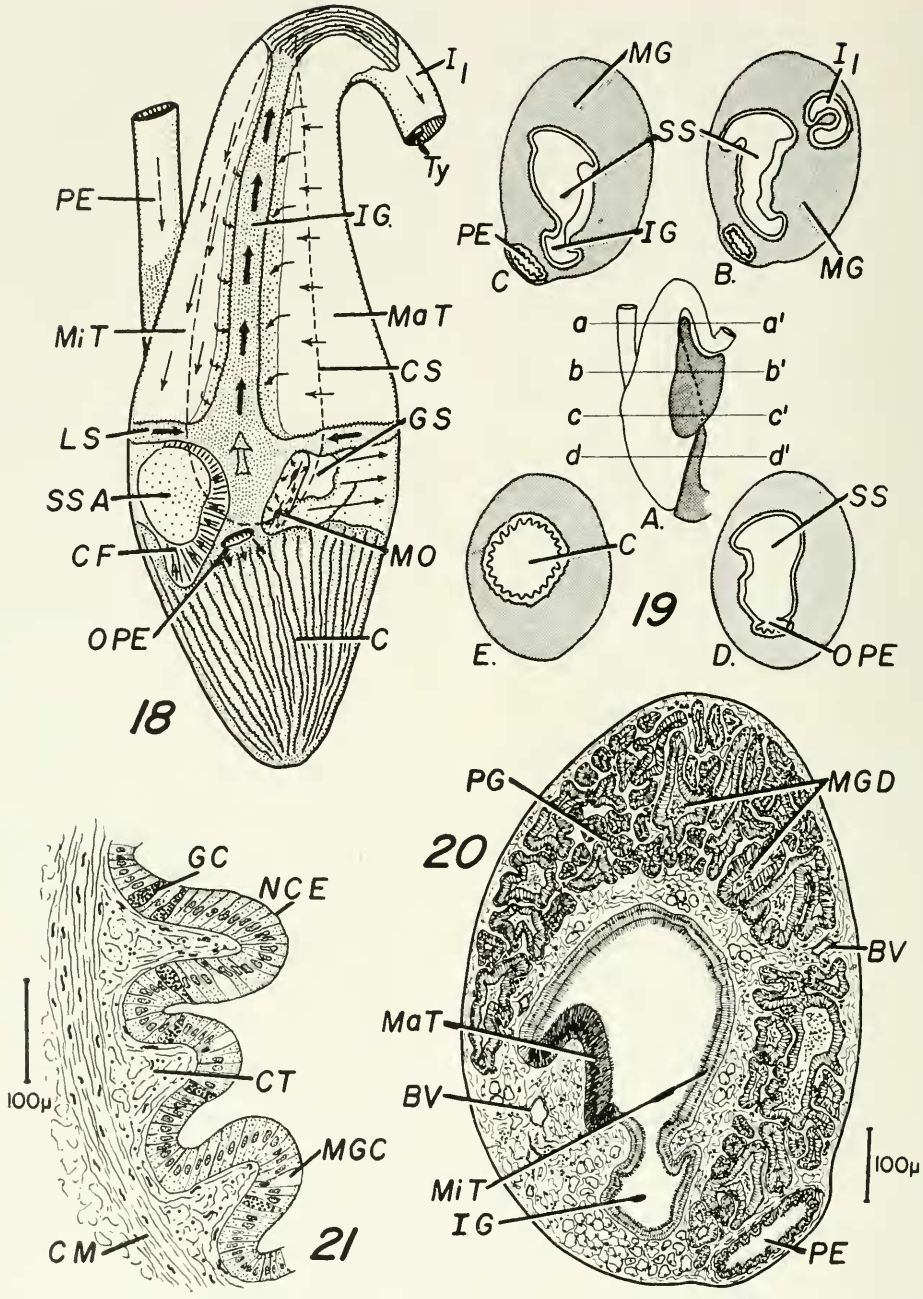


FIG. 18. Interior view of stomach (caecum and style sac), opened by a dorsal longitudinal incision and laid back slightly. Arrows indicate ciliary currents discussed in text.

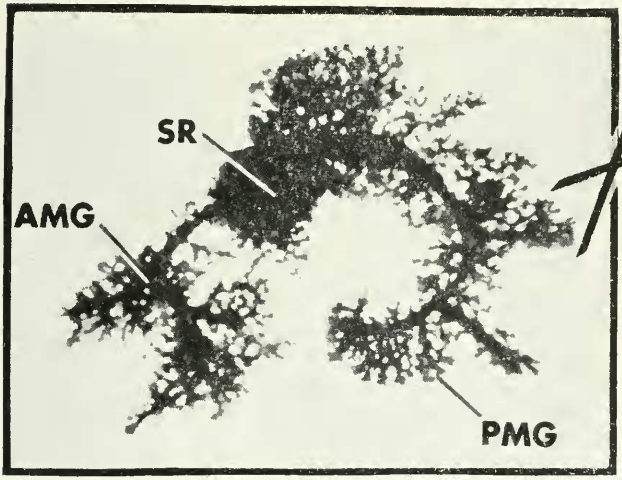
FIG. 19. Relationship of stomach to visceral mass, diagrammatic. **A.** Stomach and midgut glands, viewed dorsally. **B.-E.** Sections through levels a-a' to d-d', respectively.

FIG. 20. Cross-section through visceral mass at the mid-region of the style sac. Heidenhain's Azan.

FIG. 21. Detail of wall of caecum, in cross-section. Heidenhain's haematoxylin.

FIG. 22. Vinyl acetate injection showing a side view of the branching ductwork of the midgut glands. Most of the stomach region has been cut away for clarity.

FIG. 23. Vinyl acetate injection showing in more detail a top view of the secondary duct system indicated in Fig. 22.



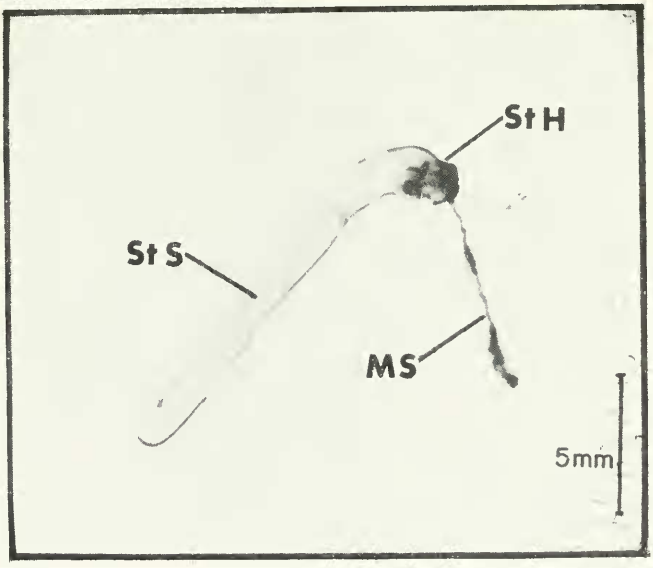


FIG. 24. A crystalline style removed from the style sac of *Nassarius obsoletus*.

TABLE 1. Histochemical affinities of various components of the digestive system.

	Coupled Tetrazonium	DMAB-Nitrite	PAS	Diastase	Toluidine Blue γ-metachromasia	Hyaluronidase	Methylene Blue Extinction point	Alcian Blue	Dialysed Iron
Buccal cavity									
subepith. mucous cells	—	—	+	fast	+	fast	<2	+	+
Ant. esophagus									
subepith. mucous cells	—	—	+	fast	+	fast	<2	+	+
Midesophagus									
epithelial mucous cells	—	—	—	fast	+	fast	<2	+	+
granular cells	+	—	+	fast	+		>6	—	—
Postesophagus									
epithelial mucous cells	—	—	+	fast	+	fast	<2	+	+
granular cells	+	—	+	fast	—		>6	—	—
Salivary glands									
Type 1 granular cells	+ +	+ +	+	fast	—		>6	—	—
Type 2 granular cells	+	+	+	fast	—		>6	—	—
Mucous cells	—	—	—		+	fast	<2	+	+
Valve of Leiblein									
" Ring " cells	—	—	—		+	fast	<2	—	+
Pseudostratified layer	—	—	—		—		<2	+	+
Gland of Leiblein									
Granular septal cells	+	—	+	fast	—		>6	—	—
Free granular cell fragments	+	—	+	fast	—		>6	—	—
Septal mucous cells	+	—	+	fast	+	fast	<2	+	+
Mucous cells of ventr. groove	+	—	+	fast	+	fast	<2	+	+
Midgut gland									
Triangular cells	—	—	—		—			—	—
Mucous cells	—	—	+	fast	+	fast	<2	+	+
Caecum									
Epithelial mucous cells	+	—	+	fast	+	fast	<2	+	+
Granular cells	+	—	+	fast	—		>6	—	—
Style sac									
Major typhlosole	+	—	+	fast	—		2-3.5	—	—
Minor typhlosole	—	—	—		—			—	—
Epithelial mucous cells	—	—	+	fast	+	fast	<2	+	+

TABLE 1.—(contd.)

	Goupled Tetrazonium	DAMB-Nitrite	PAS	Diastase	Toluidine Blue γ-metachromasia	Hyaluronidase	Methylene Blue Extinction point	Alcian Blue	Dialysed Iron
Crystalline style	+	+	+	fast	—	fast	2-3.5	+	+
Gastric shield	+	—	+	fast	—		2-3.5	—	—
Intestine (1)									
Epithelial mucous cells	—	—	—		—	fast	<2	+	+
Intestine (2)									
Epithelial mucous cells	—	—	—		+	fast	<2	+	+
Rectum									
Epithelial mucous cells	—	—	—		+	fast	<2	+	+

lacking the conspicuous granules. This type of cell in the caecum differs from those in the esophagus, however, in being devoid of cilia. The subepithelial tissue structure more closely resembles that of the postesophagus than that of the mid-esophagus. Underlying the basement membrane immediately below the epithelium is an area composed of connective tissue with a small amount of irregularly oriented muscle fibers (CT). In this area also are to be found numerous hemocytes. These blood cells have never been observed in the lumen of the caecum or between the cells of the lining epithelium. Beneath the layer of connective tissue a very heavy layer of circular muscle fibers (CM) envelops the entire caecum. No longitudinal muscle layer is present.

The lining of the style sac region of the stomach stands in sharp contrast to that of the caecum. The minor typhlosole (Fig. 20, MiT) and roof of the stomach are composed of a layer of ciliated simple columnar epithelium. The oval nuclei are uniformly located 1/3 of the distance from the base of the cells, and the cytoplasm exhibits a moderate uniform acidophilia. The epithelium of the intestinal groove (IG) consists mainly of a similar ciliated columnar epithelium which on the floor of the groove shortens to an almost cuboidal shape. In addition, goblet cells containing a PAS-positive acid mucopolysaccharide are scattered throughout the sides and floor of the groove. The epithelium of the major typhlosole (MaT) is conspicuous, being entirely made up of much taller, exceedingly thin ciliated cells. These cells also differ in having elongate very dense nuclei and, perhaps most noteworthy, a cytoplasm exhibiting pronounced basophilia. Histochemical tests indicate that the cytoplasm of these cells contains copious amounts of glycoprotein. This glycoprotein is more or less evenly distributed throughout the cells and is not confined to discrete granules.

Underlying the lining epithelium of the style sac is a thick layer of loose connective tissue containing numerous hemocytes, a few irregularly arranged muscle fibers, and blood vessels (Fig. 20). Also lying in this loose connective tissue be-

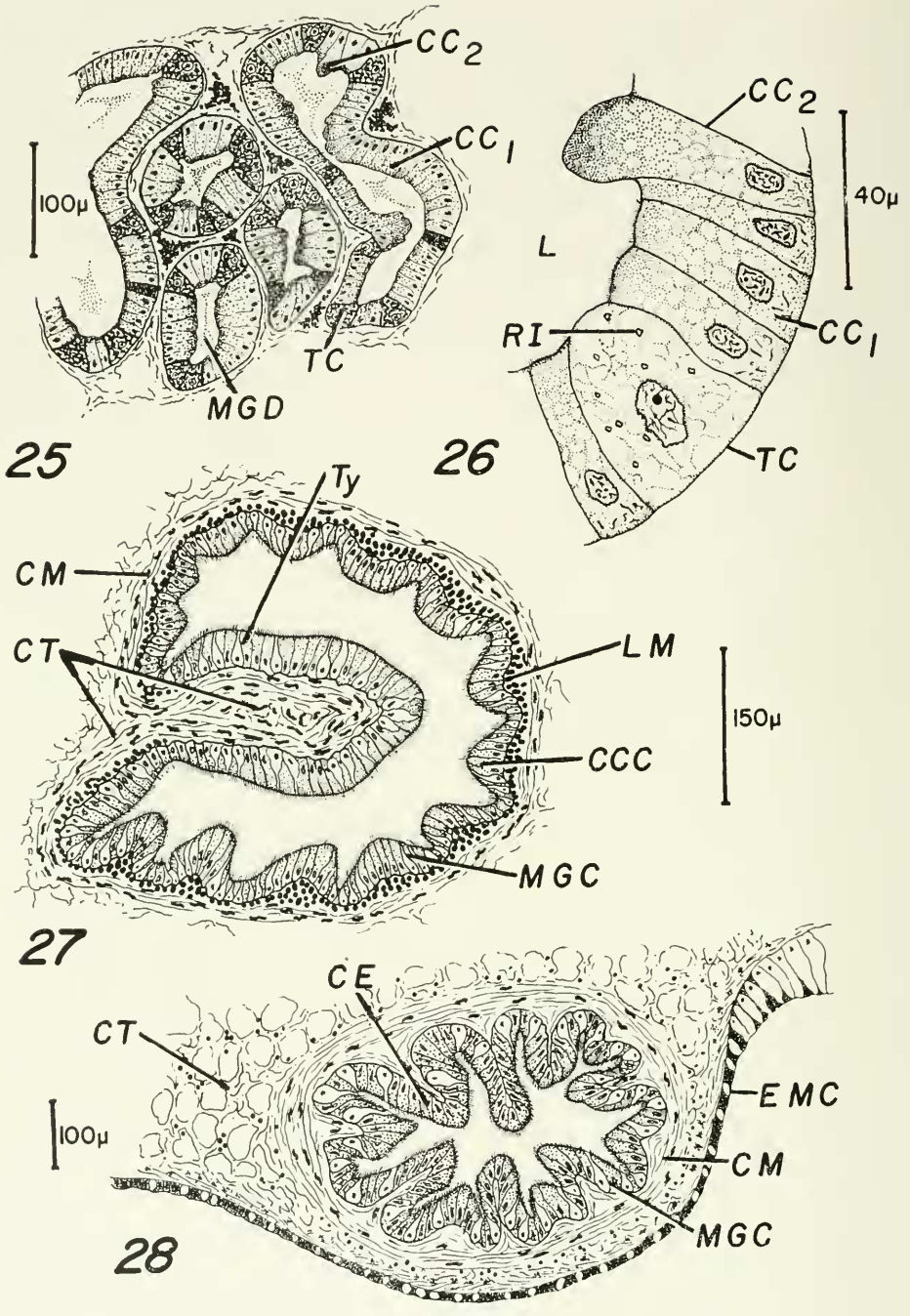


FIG. 25. Section through portion of midgut gland tissue. Heidenhain's Azan.

FIG. 26. Detail of midgut gland tubule. Epon, Azure B bromide.

FIG. 27. Cross-section through proximal region of intestine. Heidenhain's Azan.

FIG. 28. Cross-section through the distal region of the intestine. Heidenhain's Azan.

tween style sac and midgut gland are two aggregations of large cells with basal nuclei which contain large amounts of calcium within them. Indeed, these are the only sites in the visceral mass of the snail which contain calcium in sufficient amounts to be avidly stained by Nuclear fast red.

Midgut glands: The 2 midgut glands constitute the greatest bulk of the visceral mass (Fig. 1, AMG, PMG). The anterior gland forms a cradle ventral and lateral to the style sac, starting approximately at the level of the anterior duct into the stomach and proceeding anteriorly to the region of the sigmoid curve of the intestine. The posterior gland begins at its duct into the stomach anteriorly, accompanies the caecum on its right ventrolateral border, and continues spiraling toward the apex of the shell along with the gonad. The midgut glands have an acinar structure and are permeated by a tree-like network of ducts (Figs. 22 and 23).

In cross sections of the visceral mass, the tubules of the midgut glands are cut in both cross and longitudinal sections. There are 4 types of cells distinguishable in the midgut gland tubules. First, there are mucous cells, containing a PAS-positive acid mucopolysaccharide, present in very small numbers (less than 1%) scattered at random within the tubules. A 2nd type of cell is present at the angles of the tubules. These cells are usually of a triangular shape with a large subcentral nucleus containing a prominent nucleolus (Figs. 25 and 26, TC). The cytoplasm is highly vacuolated and slightly, but uniformly, acidophilic. This cell type, in addition, becomes stained throughout the cytoplasm by the DMAB-nitrite technique for tryptophan. Scattered within the apical 3/4 of the cytoplasm are small yellow refractile inclusions (RI) which do not take up the usual cytoplasmic dyes. The apical cytoplasm normally bulges slightly into the lumen of the tubule.

The cell type most prevalent (approximately 85%) is a simple columnar cell with an oval nucleus located uniformly 1/3 of the distance from the base of the cells (Figs. 25 and 26, CC_1). This cell type has a frothy appearance subapically, the cytoplasm being acidophilic throughout most of the cell but becoming lightly basophilic at the luminal edge. The prussian blue staining technique indicates the presence of iron scattered throughout the cytoplasm. The apex of the cell is covered with short microvilli which were clearly visible only in Epon sections. The fourth cell class is very similar to the preceding, except that the apex of the cell dilates into the lumen of the tubule and is devoid of microvilli (CC_2). Nearly the entire apical expansion is strongly basophilic. The latter 2 cell types in all probability represent activity stages of a single class of cells. Nothing resembling food vacuoles could be found in these cells, either in animals which had just been taken from the field or in animals which had been maintained on any of the experimental diets.

Between the ramifying tubules of the midgut gland loose connective tissue, blood vessels, numerous hemocytes, and aggregations of dark brown to black pigment granules are present (Figs. 20 and 25).

Intestine: Proceeding anteriorly from the style sac, the intestine goes but a short way before taking a sharp right-hand bend which signals the beginning of the transverse sigmoid curve (Fig. 1). This curving portion of the intestine crosses the visceral mass just posterior to the kidney and forms the forward boundary of the anterior midgut gland. After completing the sigmoid curve, the intestine arches dorsally within the right side of the mantle tissue.

The intestine can be divided, histologically, into two distinct parts which

roughly correspond to the sigmoid portion and to the dorsally arching segment. The first part of the intestine (Fig. 27) has its walls thrown into longitudinal folds similar to the esophagus. Unlike the esophagus, however, the first part of the intestine possesses a large shelf-like typhlosole along its right wall (Fig. 27, Ty). This typhlosole is a continuation of the major typhlosole found in the style sac but is histologically very distinct from the latter. The epithelium lining the lumen of the intestine is of a simple columnar type consisting of 2 classes of cells: ciliated columnar cells (CCC) and mucous goblet cells (MGC). The ciliated cells are identical to their counterparts in the esophagus. The mucous cells contain a PAS-negative acid mucopolysaccharide with a methylene blue extinction point far below any other mucin observed. The mucous goblet cells show a great increase in number over those found in the esophageal regions, comprising approximately 35% of the cells lining the lumen of the intestine. The mucous cells of the intestinal region also show a distribution different from that found in the esophagus. In the esophagus and along the typhlosole of the first part of the intestine the mucous cells are distributed essentially at random. Along the folded walls of the intestine, in contrast, the mucous goblet cells are conspicuously confined to the regions of the furrows and are not to be found along the projecting folds. Underlying the basement membrane beneath the epithelium, a heavy layer of longitudinal muscle fibers surrounds the first part of the intestine (Fig. 27, LM). These longitudinal muscles are confined to the areas of folded epithelium and are not present in the typhlosole. Outside this layer of longitudinal fibers is a layer of circular muscle fibers which encases the entire intestinal tube and penetrates the typhlosolar fold, ultimately coming to lie directly beneath the basement membrane in this region (CM).

The longitudinal folds are continued in the second portion of the intestine; the typhlosole, however, is not present. The types and distribution of cells lining the lumen are identical with the preceding part of the intestine. The subepithelial structure of this part of the intestine differs markedly from the first part in that there is no longitudinal muscle layer underneath the lining epithelium, although a strongly developed circular muscle layer is present (Fig. 28, CM).

Rectum: The intestine terminates in a short papilla which projects freely into the pallial cavity from the right side of the mantle roof. Histologically the rectum is identical with the latter portion of the intestine.

3. Evaluation of Data

Among the described species of *Nassarius*, the general anatomical and histological features of the digestive systems are similar (see Fretter & Graham, 1962, for *N. reticulatus*; Martoja, 1964, for *N. reticulatus*, *N. corniculum*, and *N. incrassatus*; Dimon, 1905; and this study for *N. obsoletus*). The following discussion summarizes some of the salient anatomical and histological characteristics of the digestive system of *Nassarius obsoletus* in particular, and of the Nassariidae in general.

One of the striking, easily observable, features of *N. obsoletus* is the extreme length of the extended proboscis (Figs. 1 and 36). This highly-developed proboscis is characteristic of all the rachiglossan Neogastropoda (Pelseneer, 1906) and is correlated with their usually carnivorous habit (Blegvad, quoted in Yonge, 1954; Fretter & Graham, 1962; and Martoja, 1964). Although not previously considered in the present study, the radular dentition of *N. obsoletus* (figured by Dimon, 1905, p 50) shows the typical rachiglossan pattern of 1+R+1 which

is well-adapted for the tearing and rasping of soft material such as flesh.

The solid cuticular thickenings (="jaws" or "mandibles") found at the anterior end of the buccal cavity of toxoglossan and some rachiglossan neogastropods (Pelseneer, 1906; Hyman, 1967) are absent in *N. obsoletus* and apparently the other species of *Nassarius* studied. The buccal cavity of *N. obsoletus* also differs from the condition found in most other gastropods (Fretter & Graham, 1962) in possessing as the only mucous cells of the lining of this cavity, large flask-shaped gland cells located *beneath* the longitudinal muscle layer, rather than having the more typical goblet-type cells confined entirely to the epithelial layer.

These subepithelial mucous cells continue the length of the anterior esophagus and, as in the buccal cavity, they are the only type of secretory cell found in the lining tissue. The rest of the esophageal tube is characterized by the presence of goblet-type mucous cells in the lining epithelium. The anterior esophagus further differs from the rest of the esophagus by the absence of cells containing glycoprotein granules. In addition, as mentioned above, the only remnants of the primitive dorsal folds and food channel (Graham, 1939) to be found in *N. obsoletus* are in the anterior esophagus. A further notable feature of the anterior esophagus (and indeed of the esophagus in general) is that though it bears cilia along the entire lining surface, it possesses a very well-developed subepithelial muscle coat. This fact is in accord with observations made in the present study (see part 4) and earlier by Jenner (1956b) that peristalsis plays an important part in moving food along the alimentary canal.

Correlated with the extensive development of the rachiglossan proboscis is the presence of the valve of Leiblein. As Graham has emphasized (1941), it performs the extremely important function of preventing food from returning to the anterior esophagus in animals which continuously elongate and contract the anterior end of their alimentary canal during feeding. Fretter & Graham (1962, p 217) state that among the rachiglossan gastropods, ". . . in the Buccinacea the valve of Leiblein is reduced or even absent (*Galeodes*, *Semifusus*, *Busycon*)". The Nassariidae, apparently, form a consistent exception to this, for in *N. obsoletus* and all the other species of *Nassarius* illustrated, a well-developed valve of Leiblein is present (Fretter & Graham, 1962; Martoja, 1964).

The salivary glands and ducts also show the effects of the elaboration of the proboscis. In the Nassariidae, as in the rest of the Stenoglossa, the differential growth of the anterior part of the gut has "pulled" the salivary glands and ducts "through" the nerve ring, the salivary glands thereby assuming a position in front of the cerebral commissure and the salivary ducts thus becoming free of any restraint imposed by the nerve ring (Fretter & Graham, 1962). The cell composition of the salivary gland tubules appears identical in the three species of *Nassarius* which have been studied in this respect (Fretter & Graham, 1962; Martoja, 1964). The mucous secretion presumably aids in lubrication of the radular apparatus during feeding; the function of the glycoprotein granules is not clear. Basic protein secretory products are of widespread occurrence in the saliva of snails (Fretter & Graham, 1962), and in several species the presence of enzymatic activity associated with the salivary glands has been shown (proteases in *Murex* by Hirsch, 1915, and by Mansour-Bek, 1934; amylase in *Littorina* by Jenkins, cited in Fretter & Graham, 1962; and disaccharases in *Nassarius obsoletus*, this study, part 3). Whether

the proteinaceous granules are the source of the enzymatic activity remains to be shown. From the histological structure of the salivary glands and ducts it would appear that secretory pressure is responsible for moving the granules and mucus from the glandular tubules into the salivary ducts, at which point ciliary action conveys the secretory products distally to the buccal cavity. The circular muscles at the terminal ends of the salivary ducts presumably act as sphincters in helping to regulate the flow into the buccal cavity.

As shown by Graham (1941) and reviewed by Fretter & Graham (1962), the elongation of the proboscis in the Rachiglossa has been further accompanied by a "stripping off" of the glandular area associated with the midesophagus, resulting in the formation of a discrete organ, the gland of Leiblein, whose only contact with the parent midesophagus is by the duct emptying into it. Presumably, therefore, this duct from the gland of Leiblein marks the most posterior extent of the "pre-stripped" midesophagus (Graham, 1941). From the histological evidence, however, this appears not to be the case in *N. obsoletus*. As described above, a well-defined structural change occurs in the esophageal region some distance posterior to the duct from the gland of Leiblein, at the level of the columellar muscle.

That functional activity has been retained by the gland of Leiblein regardless of anatomical shifting is attested to by the conspicuous apocrine secretions reported for these glands in rachiglossans in general and in *N. reticulatus* (Martoja, 1964) and *N. obsoletus* (this study) in particular. Further evidence for a functional role for the gland of Leiblein is given by the repeated demonstration of digestive enzyme activity in its secretion (Hirsch, 1915; Mansour-Bek, 1934; Brock, 1936; this paper part 3).

The stomach of *N. obsoletus* is comparable with those of other rachiglossans in the assumption of a sac-like shape, in showing a migration of the esophageal opening posteriorly, and in the reduction of ciliary sorting fields to a minimum (for illustrations of other rachiglossan stomachs, see Graham, 1949, Morton, 1958b; Fretter & Graham, 1962; Martoja, 1964; and Wu, 1965). The caecum of *N. obsoletus* is apparently unique among the Nassariidae in lacking ciliation. The deep longitudinal folding undoubtedly serves the mechanical function of allowing a great deal of expansion when the snail has ingested food, thereby permitting the caecum to serve as a temporary storage organ for this material. The underlying heavy circular musculature is then responsible for moving the food mass anteriorly into the style sac region of the stomach.

The possession of a gastric shield, regarded as a primitive character in the Gastropoda, has been confirmed for all the species of *Nassarius* studied in detail (Graham, 1949; Martoja, 1964; and the present study), and for a related species, *Cyclope neritea* (Morton, 1958b). The production of a crystalline style in the "carnivorous" Stenoglossa is incompatible with the principal that "a crystalline style and the carnivorous habit cannot normally co-exist" (Yonge, 1930). Although neither Martoja (1964) nor Graham (1949) report the presence of styles in the Nassariidae studied by them, styles are definitely present in *Cyclope neritea* and *Nassarius obsoletus*. Whether or not these styles are truly functional (*i.e.*, as repositories of enzymes) or merely neomorphic protostyles derived from food string aggregations, is not known for *Cyclope*, but it has been shown (this study, part 3) that styles of *N. obsoletus* do indeed exhibit enzymatic activity.

With regard to the cellular composition

of the midgut glands, it is apparent that the histology of the midgut gland varies considerably from animal to animal amongst the prosobranchs (Fretter & Graham, 1962). The types and structure of midgut gland cells herein described for *N. obsoletus* are in good agreement with those described by Martoja (1964) for the European species of *Nassarius*. Little attempt will be made here to relate the cell types found in *N. obsoletus* with those found in the rest of the Gastropoda. The difficulties and pitfalls of synonymy for even a single genus are well illustrated in Sumner's thorough review (1965) of the midgut gland cells of *Helix*. Nevertheless, the triangular cells of *N. obsoletus* agree well with the " cellules coniques " of Martoja (and, in general, with the " secretory cells " of Fretter & Graham) and the columnar cells (types, or phases, 1 and 2) with Martoja's " cellules cylindriques " (and Fretter & Graham's " digestive cells ").

The intestine, as previously shown, is characterized by a regional differentiation due to the presence or absence of a typhlosole and to differences in the subepithelial muscle coat. A great increase in the number of mucous cells in the intestine as compared with the esophagus is also a conspicuous feature. The latter 2 characteristics, in particular, are undoubtedly correlated with consolidation of the feces and movement of material through the intestinal lumen (see further discussion of this below, in part IV and general discussion).

In view of Martoja's conclusion (1964) that the amoebocytes of the European species of *Nassarius* play a very important role in the digestion of food, it is noteworthy that in *N. obsoletus* no histological evidence could be observed that the amoebocytes (hemocytes) were ever present within the lumen of the gut or even between the epithelial cells lining the various regions of the alimentary canal.

Finally, the digestive tract of *N. obsoletus*, like those of all gastropods, is characterized by the abundance of mucus-secreting cells. The functional correlates of this are well-known and understood (Morton, 1958; Fretter & Graham, 1962; for reviews and extensive bibliographies). Investigations (principally histochemical) on the diversity of mucin types in molluscs are still in their infancy. Those studies which have been made (for example by Martoja, 1964, on the digestive systems of certain of the Nassariidae, and by Smith, 1965, on the reproductive tract of the slug *Arion ater*) indicate that great diversity and regional differentiation of mucin types is the rule, even within a single organ system. The following histochemically-detected classes of mucins are conspicuously present in the digestive system of *Nassarius obsoletus*: (1) PAS-positive acid mucopolysaccharides of epithelial goblet cells: in midesophagus, postesophagus, ventral groove of gland of Leiblein, septal cells of gland of Leiblein, caecum, midgut gland, and ventral groove of style sac: (2) PAS-positive acid mucopolysaccharides of subepithelial unicellular gland cells: in buccal cavity and anterior esophagus; (3) PAS-negative acid mucopolysaccharides of epithelial goblet cells: in 1st and 2nd part of the intestine and rectum; (4) PAS-negative acid mucopolysaccharides in gland cells of salivary tubules: (5) DMAB-nitrite positive glycoproteins in gland cells of salivary tubules; (6) DMAB-nitrite negative glycoproteins in epithelial columnar cells: in midesophagus, postesophagus, and caecum; (7) Histochemically problematical mucins in valve of Leiblein: and (8) glycoprotein/acid mucopolysaccharide material of the crystalline style. Thus, the present histochemical study adds further evidence for the preponderance of mucin heterogeneity in gastropod organ systems.

III. ENZYME HISTOCHEMISTRY

1. Materials and methods

All animals used in this part of the study had been maintained at the University of Michigan in sea-water aquaria prior to fixation. Tissues were quick-frozen by quenching in isopentane cooled by liquid nitrogen and then sectioned at 8–12 microns on an International model CT cryostat equipped with a razor blade holder. Fixation, either before or after sectioning, was carried out in Lillie's buffered neutral formalin at 4°C for 1 hour.

Acid phosphatase: Two methods were employed for the detection of this enzyme. The first was Gomori's lead nitrate method (Gomori, 1950) on post-fixed material with sodium β-glycerophosphate as substrate. The other was the simultaneous azo dye method (Barka & Anderson, 1963) on prefixed material. In this method, sodium α-naphthyl acid phosphate was the substrate and freshly diazotized pararosanilin was used as coupler. The reaction was carried out at room temperature in barbiturate buffer (pH 6·0). Results from both Gomori and simultaneous coupling techniques were in complete agreement.

Alkaline phosphatase: As in the preceding case, 2 different methods were employed. These were the Gomori (1952) calcium-cobalt method and the simultaneous coupling technique as given in Barka & Anderson (1963). In the former, sodium-β-glycerophosphate was used as substrate on post-fixed tissue. Sodium α-naphthyl acid phosphate was employed as substrate in the latter, with Fast red TR as diazo coupler. This reaction was carried out on prefixed material at pH 9·2 in barbiturate buffer. As before, results obtained from the Gomori and simultaneous coupling methods were in agreement.

Esterase: The indoxyl acetate method for nonspecific esterases was employed, according to the method of Holt & Withers (1952) and Holt (1958). Prefixed tissue sections were incubated at 37°C. in O-acetyl-5-bromoindoxyl. The incubating medium was maintained at pH 6·5 with tris (hydroxymethyl) aminomethane buffer, and the enzyme activity was rendered visible by the formation of insoluble indigo with ferricyanide-ferrocyanide as the redox pair.

Cathepsin C: This method, developed by Hess & Pearse (1958), utilizes indoxyl acetates as substrate (O-acetyl-5-bromoindoxyl was used in the present study). The indoxyl liberated by enxymatic hydrolysis is converted, as in the previous method, to indigo by ferricyanide-ferrocyanide oxidation. The specificity for cathepsin C is achieved by preincubation of all sections in E-600 (diethyl-p-nitrophenyl phosphate) which inactivates all B-type esterases present in the tissues. Sections subsequently incubated in activator (1×10^{-3}M cysteine) and inhibitor (1×10^{-3}M lead nitrate) are compared with control sections, and any cell, containing indigo in the control section, which contains more indigo after incubation with the activator and less after the use of the inhibitor, is considered to exhibit esterase activity of the type associated with cathepsin C.

Leucine Amino Peptidase: The simultaneous coupling method of Nachlas, *et al.* (1957) using L-leucyl-β-napthyl amide as substrate was employed. The coupler used was Fast blue B salt and the reaction was carried out in acetate buffer (pH 6·5) at 37°C.

Beta-glucuronidase: This enzyme was detected by the post-coupling method of Seligman, *et al.* (1954). The synthetic substrate used was 6-bromo-2-naphthyl-β-D-glucuronide. Sections were incubated in phosphate-citrate buffer (pH 4·9) at 37°C. and Fast blue B salt was used as the diazo coupler.

Gomori's Tween method for lipase (Pearse, 1960), using Tween 80 (polyoxyethylene sorbitan monooleate) as substrate, was performed on the various tissues, but was eventually abandoned because only patchily-distributed nonspecific staining could be obtained. Post-coupling techniques for β-glucosidase and β-galactosidase (Rutenberg, *et al.* 1958) using 6-bromo-2-naphthyl glycosides were likewise abandoned because of failure to achieve consistent results.

Sections incubated in medium lacking substrate and pre-incubation of sections in water at 95°C for 5 minutes were used as controls for all staining procedures.

2. Results

Buccal cavity: The only enzyme demonstrable in the buccal cavity was a nonspecific esterase. Enzyme activity was present in the entire lining epithelial layer, although it was not localized identically in every cell. All cells exhibited enzyme activity at their apical regions, while more basal activity varied among the cells from none at all to complete and even distribution throughout the entire cytoplasm.

Anterior esophagus: Enzymes demonstrable in the anterior esophagus included acid phosphatase, esterase, and leucine amino peptidase. The acid phosphatase activity was confined to the apices of the cells lining the lumen of this region. The non-particulate homogeneous reaction product formed a distinct continuous band immediately beneath the cilia. No particulate reaction sites in the cytoplasm of these cells were observed. Esterase activity was scattered along the epithelium, rather than exhibiting the continuity observable in the buccal cavity epithelium. The cellular distribution, however, was similar to that found in the buccal cavity epithelial cells—being present apically in all the cells exhibiting activity, but varying to the extent to which it extended into the basal cytoplasm. Leucine amino peptidase activity was found in all cells in the epithelial lining. Activity was confined to the distal 2/3 of the cells and the reaction product was present as a homogeneous precipitate throughout this area.

Midesophagus: Enzymes demonstrable in the midesophagus include acid phosphatase, alkaline phosphatase, esterase, and leucine amino peptidase. The reaction product of acid phosphatase activity was confined to a thin homogeneous layer at the luminal border of the epithelium, as in the anterior esophagus. Alkaline phosphatase activity was similarly localized along the margin of the lining epithelial cell layer. In both, no activity was observed deeper within the cell cytoplasm. Esterase activity was again scattered throughout the epithelial lining and intracellular localization was varied, as before. Leucine amino peptidase activity was present homogeneously throughout the apical 2/3 of the cells lining the midesophagus.

Postesophagus: Enzymes present in the postesophageal epithelium included acid phosphatase, alkaline phosphatase, and esterase. The distribution of these enzymes in the lining epithelial cells was identical to that described above.

Valve of Leiblein: The only enzyme demonstrable in the valve of Leiblein was acid phosphatase. The sites of localization were in the ciliated columnar epithelial cells which are directly continuous with the lining epithelia of the anterior and midesophagus, and in the cells which make up the "ring" surrounding the cone-shaped papilla. Activity in the ciliated cells was confined, as before, to the luminal border. In the cells of the "ring", however, the reaction product was deposited homogeneously throughout the entire cytoplasm. No enzyme activity was observed in the pseudostratified portion of the valve of Leiblein.

Gland of Leiblein: Enzymatic activity in the gland of Leiblein was demonstrable for alkaline phosphatase, acid phosphatase, and leucine amino peptidase. Acid and alkaline phosphatase activity was present in some, but not all, of the septal cells. In those cells in which activity was found, the reaction product was confined to the apical borders of the cells. Very strong leucine amino peptidase activity was present in all the septal cells. The reaction product was deposited homogeneously throughout the cytoplasm of these cells and not restricted to a particular portion of them.

Salivary glands: No enzymatic activity could be demonstrated by any of the histochemical techniques employed.

Caecum: Enzymes detectable in the epithelium lining the caecum included acid phosphatase, alkaline phosphatase, and esterase. Localization of these enzymes was identical to that of the post-esophagus.

Style sac: Alkaline phosphatase, acid phosphatase, and esterase were demonstrable in the epithelium lining the style sac. The distribution of phosphatase activity was as follows: a very thin homogeneous band of activity appeared at the luminal border along the roof of the style sac; contrasting sharply with this at the regions of the minor typhlosole and ventral groove was a thick band of much greater activity which extended below the apices of the cells into the cytoplasm. The reaction product deposited in this thick band of enzyme activity was also homogeneous. No activity could be detected in the basal cytoplasm of these cells. In the cells covering the major typhlosole, no phosphatase activity whatsoever could be demonstrated. Esterase activity was confined to the regions of the roof of the style sac, the minor typhlosole, and the ventral groove.

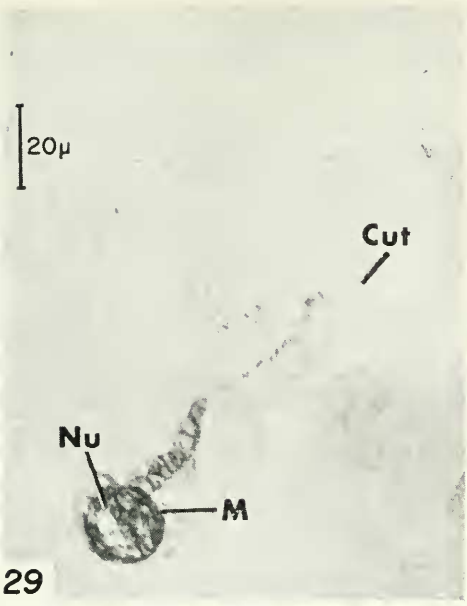
Midgut gland: Enzymes demonstrable in the midgut gland included alkaline phosphatase, acid phosphatase, esterase, cathapsin C, and β-glucuronidase. Leucine amino peptidase activity could not be detected. Phosphatase activity, as in the previous tissues, was confined to a thin band on the luminal border of the midgut gland tubules (Figs. 30 and 31). Not all cells gave the reaction, but apparently there was no strict correlation with cell type, as both the triangular cells and the columnar cells exhibited activity. Strong esterase activity was shown by the cells of the midgut gland tubules. This activity was spread throughout the cytoplasm (Fig. 32). Beta-glucuronidase activity was found throughout the cells of the midgut gland tubules. Cathepsin C activity was found scattered throughout the epithelial lining of the tubules. This enzyme was apparently confined to the columnar cells, being most noticeable in type 2 cells which bulge into the lumina of the ducts. The intracellular localization was homogeneous throughout the cytoplasm of the cells.

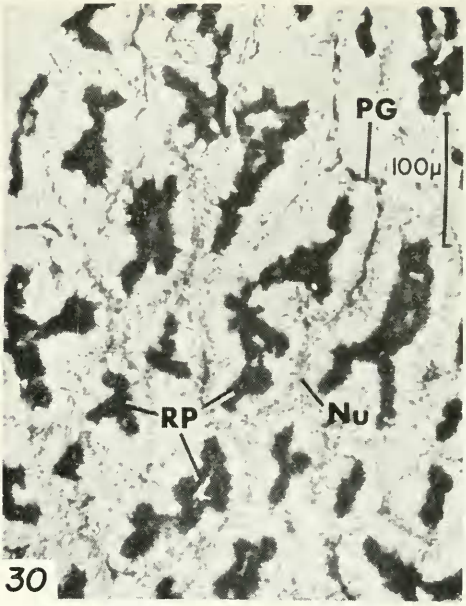
Intestine: In both regions of the intestine, acid phosphatase, alkaline phosphatase, and esterase could be demonstrated. The activity and distribution of these enzymes was essentially identical to that found in the caecum and esophagus. Additionally, in the second part of the intestine, leucine amino peptidase could be detected in the epithelial lining. Activity of this enzyme was spread throughout the apical 2/3 of the cells.

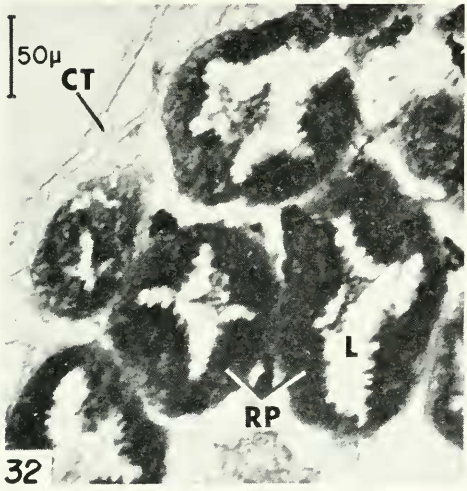
Rectum: In the rectum, no enzymatic activity could be detected by any of the techniques employed.

3. Evaluation of data

Although a large literature has accumulated on the histochemical localization of hydrolytic enzymes (principally in vertebrate tissues), the biological significance (or functional role) correlated with the observed enzymatic distribution is in most cases not well known. Few specific







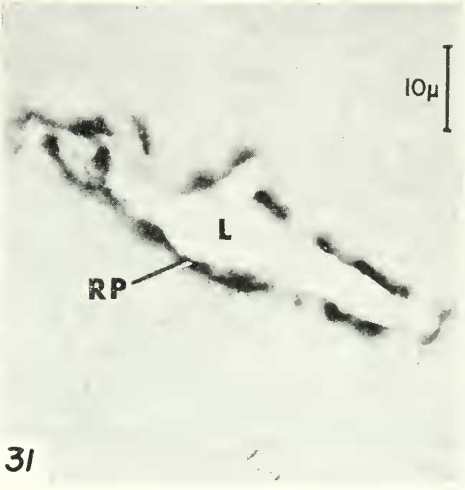


FIG. 29. Subepithelial mucous gland cell within wall of buccal cavity. PAS technique.

FIG. 30. Midgut gland tubules showing a heavy deposit of acid phosphatase reaction product along the lumenal borders. Gomori lead nitrate method with Mayer's haemalum counterstain.

FIG. 31. Cross-section of single midgut gland tubule showing acid phosphatase localization confined to cell apices. Simultaneous coupling method, no counterstain.

FIG. 32. Midgut gland tubules showing strong esterase activity throughout the cells. Indoxyl acetate method.

conclusions, therefore, can be drawn concerning the histochemical data just presented (see Table 2 for tabulated results).

The presence of alkaline phosphatase along the free cell borders of the epithelia in *Nassarius obsoletus* is consistent with

TABLE 2. Enzyme histochemistry of various components of the digestive system.

	Acid phosphatase	Alkaline phosphatase	Esterase	Cathepsin C	β-Glucuronidase	Leucine amino peptidase
Buccal cavity						
Epithelial cells	−	−	−	−	−	−
Ant. esophagus						
Epithelial cells	+	−	+	−	−	−
Midesophagus						
Epithelial cells	−	−	−	−	−	+
Postesophagus						
Epithelial cells		+	+	−	−	−
Salivary glands						
Granule cells	−	−	−	−	−	−
Valve of Leiblein						
"Ring" cells		−	−	−	−	−
Epithelial cells		−	−	−	−	−
Gland of Leiblein						
Septal cells	−	−	−	−	−	+
Midgut gland						
Columnar cells		+	−	−	−	−
Haemocytes	−	−	−	−	−	−
Caecum						
Epithelial cells	+	+	+	−	−	−
Style sac						
Roof epithellium	+	+	+	−	−	−
Major typhlosole	−	−	−	−	−	+
Minor typhlosole	+	+	+	−	−	−
Ventral groove	+	+	+	−	−	−
Intestine (1)						
Epithelial cells	−	−	−	−	−	−
Intestine (2)						
Epithelial cells		+	−	−	−	+
Rectum						
Epithelial cells	−	−	−	−	−	−

the localization found in many vertebrate tissues. Although clear-cut evidence of a specific functional role is lacking for alkaline phosphatase, the nearly universal association of this enzyme with especially active cell surfaces (such as those possessing microvilli) is taken to indicate that it participates in the movement of mole-

cules across the cell membrane (Rothstein, *et al.*, 1953).

The membrane-associated acid phosphatase and non-specific esterase likewise are thought to act in the transport of material into the cell (Richardson, *et al.*, 1955). Acid phosphatase, an enzyme which has been clearly shown to be associated with lysosomes (deDuve, 1959), was not found in the subapical cytoplasm of the midgut gland cells of *Nassarius obsoletus*. This finding is of special interest in view of the current concept of the mechanism of intracellular digestion in which lysosomes play a central role (deDuve & Wattiaux, 1966). This apparent lack of a significant lysosomal component correlates well with the histological picture using conventional procedures in which no evidence of food-vacuole formation could be observed.

The presence of especially strong esterase activity throughout the midgut gland cells may well reflect a metabolic role rather than a purely digestive one, for it is well established that, in addition to hydrolytic activity, esterases are capable of participating in synthetic reactions as well as mediating replacement of ester components, the latter process being known as transesterification (Hofstee, 1960).

Esterase activity of the type associated with cathepsin C was found in the columnar cells of the midgut glands. As stressed by Tallan, *et al.*, (1952), intracellular catheptic activity results from a whole family of enzymes rather than a single proteinase. Cathepsin C has been shown to be an organophosphate-resistant member of this family which is homospecific with chymotrypsin with regard to substrate specificity. Again, the physiological role of this enzyme is not clear; it is believed, however, that catheptic activity plays a role in the biosynthesis of the peptide bonds of proteins and of naturally occurring peptides (Fruton & Simmonds, 1958).

Especially strong leucine amino peptidase activity was found in the gland of Leiblein septal cells and in the epithelial cells lining the second part of the intestine. Its presence in the gland of Leiblein may well be correlated with the secretory activity of that organ, but its presence in, and restriction to, the posterior region of the intestine is of unknown significance.

Beta-glucuroridase activity was found to be present in the midgut gland of *N. obsoletus*. This enzyme is one of the hydrolytic enzymes also known to be often linked with lysosomal particles (deDuve, 1959; deDuve & Wattiaux, 1966) although in the midgut gland cells of *N. obsoletus*, the enzymatic activity was distributed throughout the entire cytoplasm. The presence of β-glucuronidase has been histochemically rendered visible in gastropod tissue previously (Billet & McGee-Russell, 1955, in *Helix*), and in a survey study, Dodgson, *et al.*, (1953) have biochemically demonstrated its presence in a number of marine gastropods including the rachiglossans *Nucella lapillus* and *Buccinum undatum*. These latter investigators concluded that, on the basis of the variety of gastropods which possessed β-glucuronidase activity, there was apparently no strict correlation with habitat or feeding preferences. They did point out, however, that it was possible that the enzyme plays a digestive role, inasmuch as many of the marine algae on which some of these snails feed contain polysaccharide material rich in uronic acid residues. This type of functional role appears very probable for the β-glucuronidase of *Nassarius obsoletus*.

IV. *IN VITRO* ENZYME ANALYSES.

1. Materials and methods

Preparation of tissues: All tissues used were from recently collected snails which

were maintained in running seawater aquaria at the Marine Biological Laboratory, Woods Hole, Massachusetts. The shells of the snails were gently cracked using a " C "-clamp and the soft parts removed *in toto* by grasping the columellar muscle with a pair of watchmaker's forceps. The tissues investigated were carefully dissected out under a stereomicroscope. Only posterior midgut glands were used, as these could be freed most cleanly from adjacent tissues (stomach caecum and gonad). The gland of Leiblein and salivary glands could be cleanly separated from their adjacent organs, the esophagus and cerebral ganglia respectively. The excised tissue was then quickly rinsed in cold distilled water and placed in cold (O°C) molluscan Ringer's solution without buffer (Cavanaugh, 1956). The cold tissues were subsequently homogenized at low speed in a glass tissue grinder with a teflon pestel and the resulting homogenate was allowed to stand in the cold for 1 hour with intermittent agitation. The preparation was then centrifuged for 10 minutes at *ca.* 3000 rpm to remove the larger unsuspended particles. The supernatant was decanted and assayed for enzymatic activity.

Crystalline styles were removed from animals, quickly rinsed in cold distilled water and only those portions of the styles containing no obvious debris allowed to dissolve in cold molluscan Ringer's. Stomach fluid was obtained by making a slit in the caecum where it lies adjacent to the surface of the visceral mass and inserting a fine-tipped Pasteur pipette into the lumen. Special care was taken to insure that no midgut gland material was inadvertently picked up. The stomach fluid was immediately put into cold Ringer's solution. In an effort to eliminate bacterial contamination, both the crystalline style and stomach fluid preparations were then filtered through a 0·22 micron Millipore filter held by a Swinnex filter apparatus (both from Millipore Filter Corp., Bedford, Mass.). The resulting solutions were assayed for enzymatic activity.

Determination of enzymatic activity: Disaccharase, amylase, and cellulase activities were estimated by measuring the liberation of glucose from the various substrates. The reaction mixture for the disaccharase determinations contained 1·0 ml enzyme preparation, 10 micromoles of sugar (maltose, cellobiose, sucrose, melibiose, or lactose) and 100 micromoles of buffer, made up to a final volume of 2·0 ml. The reaction mixture for the amylase determinations contained 1·0 ml enzyme preparation, 0·1 mg starch or glycogen and 100 micromoles buffer, made up to a final volume of 3·0 ml. The reaction mixture for cellulase determinations was identical to those for amylase determinations with sodium carboxymethyl cellulose as substratum. Phosphate buffer was employed in the experiments, at pH 6·0 for the disaccharases, and at pH 7·0 for the amylases and cellulases. All reactions were run at 20°C from 2 to 24 hours. Toluene was added to the reaction mixtures to inhibit bacterial activity on all runs over 3 hours. Reactions were stopped by the addition of equimolar amounts of $Ba(OH)_2$ and $ZnSO_4$ according to the method of Weichselbaum & Somogyi (1941). Glucose in the protein-free supernatant was determined with the " Glucostat " reagent, with the exception that the reagent was dissolved in 0·25 M tris (hydroxymethyl) —aminomethane—HCl buffer instead of phosphate. This modification has been introduced (Dahlqvist, 1961) to inhibit maltase present in commercial preparations of glucose oxidase. Control tubes containing only tissue preparations, or only substrate, in buffer, were run simultaneously with all experimental mixtures, and enzymatic activity was taken as the difference in the amount of glucose in the

experimental tube and the amount of glucose in the control tubes. One ml samples of the enzyme preparations were precipitated with trichloroacetic acid (TCA) at a final concentration of 5% and set aside for tissue protein determinations. Colorimetric determinations on all experiments were made with a Coleman Jr. spectrophotometer.

Esterase and lipase activity were estimated by the method of Seligman & Nachlas (1963) in which 2-naphthol liberated from 2-naphthyl laurate is coupled with tetrazotized 0-dianisidine to give a purple azo dye which is then extracted with ethyl acetate and determined colorimetrically. The reaction mixture consisted of 1·0 ml enzyme preparation, 500 micromoles buffer, 10 micrograms substrate, with or without 1·0 ml 8×10^{-2} M sodium taurocholate, made up to a final volume of 7·0 ml. The buffers used were phthalate-NaOH at pH 5·5 and 6·0; phosphate at pH 5·5, 6·0, 6·5, 7·0, 7·25, 7·5, 7·75, and 8·0; barbiturate-HCl at pH 8·0, 8·25, 8·5 and 9·0; and glycine-NaOH at pH 9·0, 9·5 and 10·0. All reactions were run at 20°C for 2 hours and stopped by the addition of TCA to give a final concentration of 5%. One ml samples of the enzyme preparations were precipitated with TCA and set aside for tissue protein determinations. Control tubes containing only tissue preparations, or only substrate, in buffer, were run simultaneously with all experimental mixtures and esterase activity was taken as the difference in the amount of 2-naphthol in the experimental tubes lacking taurocholate and the amounts of 2-naphthol in the control tubes. Similarly, lipase activity was taken as the amount of 2-naphthol liberated in the presence of taurocholate in excess of the total amount in the tubes lacking taurocholate and the control mixtures.

Protease activity was estimated by measuring the liberation of TCA-soluble protein from TCA-insoluble protein (casein and bovine serum albumen). The reaction mixture consisted of 1·0 ml enzyme preparation, 0·1 mg substrate, and 100 micromoles of phosphate buffer (pH 6·0 and 8·0), made up to a final volume of 3·0 ml. Control tubes containing only tissue preparations, or only substrate, in buffer, were run simultaneously with all experimental mixtures. Protein in this assay as well as the protein in all preceding assays was measured by the method of Lowry, *et al.* (1951).

2. Results

Enzymatic cleavage of disaccharides: Table 3 shows the distribution of disacharase activity in the organs examined. It can be seen that maltose and cellubiose were hydrolyzed by all the tissues tested, with highest activity recorded for the crystalline style, stomach fluid, and gland of Leiblein. Midgut gland preparations were able to hydrolyze the α- and β-galactosides in low amounts and the stomach fluid also had trace amounts of activity. The gland of Leiblein, in contrast, shows considerable hydrolytic activity with lactose as substrate. Invertase (sucrase) activity did not parallel maltase activity at all, rather it was found only in the midgut gland with traces of activity in the stomach fluid.

Enzymatic cleavage of polysaccharides: Hydrolysis of starch, glycogen, and sodium carboxymethylcellulose is shown in Table 4. Highest activities were found in extracts of the crystalline style and in stomach fluid with starch and glycogen as substrates. Midgut gland preparations also showed some activity with these substrates. Midgut gland preparations showed moderate activity, and stomach fluid low activity, with carboxymethylcellulose as substrate.

Esterase—lipase activity in midgut gland homogenates: The histochemical investigations reported above (part 2) had shown

TABLE 3. Disaccharase activity in *Nassarius obsoletus*. (Activity expressed as micromoles substrate hydrolyzed/gram tissue protein/hour. All experiments run at pH 6·0 at 20° C.)

Organ	Maltose	Cellobiose	Sucrose	Melibiose	Lactose
Salivary glands	230	58·8	nil	nil	nil
Gland of Leiblein	420	1860	nil	nil	563
Crystalline style	3800	36·0	nil	nil	nil
Stomach fluid	1710	59·0	trace	trace	trace
Midgut gland	287	66·5	278	6·94	34·7

TABLE 4. Amylase and cellulase activity in *Nassarius obsoletus*. (Activity expressed as micromoles glucose liberated/gram of tissue protein/hour. All experiments run at pH 7·0 at 20° C.)

Organ	Glycogen	Starch	Sodium carboxymethyl-cellulose
Salivary glands	nil	trace	nil
Gland of Leiblein	trace	trace	trace
Crystalline style	2650	2790	trace
Stomach fluid	1090	1170	27·0
Midgut gland	415	439	196

TABLE 5. Protease activity in *Nassarius obsoletus*. (Activity expressed as micrograms protein rendered soluble/gram of tissue protein/hour. All experiments run at pH 6·0 at 20° C.)

	Salivary glands	Gland of Leiblein	Midgut gland	Stomach kuid
TCA-soluble protein	nil	2380	260	7220

the presence of strong non-specific esterase activity in the midgut gland; the technique for demonstration of lipase by means of the Gomori Tween method, however, gave equivocal results. The method of Seligman & Nachlas (1963) was used to determine whether any differences could be detected *in vitro*

between esterase activity and lipase activity. With the technique employed it appears that there is indeed a lipase present. Maximum activities for the tissue homogenate are similar for both enzymes (803 micromoles/gram protein/hour for the esterase and 850 micromoles/gram protein/hour for the lipase). However, as the pH dependency curves show (Fig. 33), the shape of the curves and the pH optima for the enzymes are clearly different. The lipase optimum appears to be about 7·5, while that for the esterase is 8·25.

Proteolytic activity: Enzymatic hydrolysis of protein is shown in Table 5. The values given are for the maximum activity measured with casein as substrate at pH 6·0. Lower but significant activity was observed at pH 8·0 with casein as substrate, but only traces of activity were observed with bovine serum albumen as substrate, either at pH 6·0 or 8·0.

3. Evaluation of results

From the results on hydrolytic activity reported above, one can draw some reasonable, if not highly specific, conclusions about the enzymatic complement of the digestive system of *Nassarius obsoletus*.

Disaccharide and polysaccharide substrates were chosen so as to give the presumably complete set of glycosidic linkages which are thought to be of paramount importance in determining glycosidase specificity (Veibel, 1950). Thus, for maltose to be hydrolyzed, an α-glucosidase must be present; similarly for cellobiose, a β-glucosidase: for sucrose, an invertase (α-glucosidase or β-fructosidase); for melibiose, an α-galactosidase; for lactose, a β-galactosidase: for glycogen and starch, an amylo-1, 4-glucosidase; and for cellulose, a β-1, 4-glucosidase (cellulase). Since most enzyme characterizations have been done with yeast and bacteria as source materials,

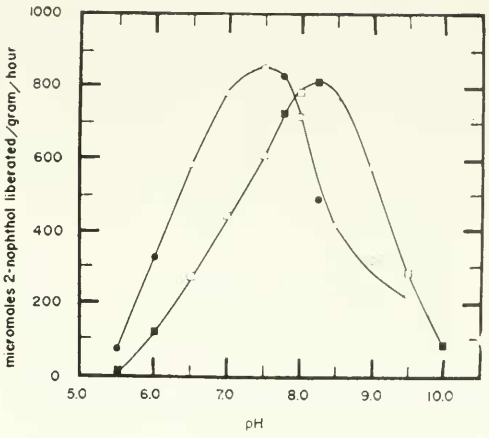


FIG. 33. Lipase-esterase pH curves from midgut gland homogenates. Circles = lipase; squares = esterase. Closed figures = single determination; open figures = mean of 3 determinations.

only general comparisons can be drawn.

Two classes of enzymes are known to act on the disaccharide sucrose, namely α-glucosidases (glucosido-invertases) and β-fructofuranosidases (Neuberg & Mandl, 1950). The animal invertases that have been sufficiently characterized, however, are all of the glucosido-invertase type (Myrback, 1960). There has been controversy over whether or not maltase and invertase (sucrase) activity results from two types of enzyme or from a single α-glucosidase with low specificity with regard to the aglucon moiety. The evidence is somewhat conflicting, but data on metazoan enzymes indicate that animal maltase is incapable of acting on sucrose (Gottschalk, 1950). From the distribution of maltase activity shown in Table 3, it appears that since tissue preparations, run simultaneously, showed maltase activity but had no hydrolytic effect with sucrose as substrate, there is a true maltase present in at least the salivary glands, gland of Leiblein, crystalline style, and stomach fluid. The finding of both maltase and sucrase action in the midgut gland (and their near equality

in activity) may indicate that there is a single relatively unspecific α-glucosidase present whose lower activity is perhaps an indication of a metabolic role rather than a purely digestive one. The site of origin of the high maltase activity in the stomach fluid and style is not clear. The organs which are known to release products into the digestive tube (salivary glands, gland of Leiblein, and midgut gland; Fretter & Graham, 1962; Hyman, 1967) appear to have too little maltase to contribute significantly to the extremely high activity found in the style. The substance of the style (principally mucoprotein) is thought to be secreted by the typhlosoles of the style sac and perhaps these structures are also responsible for secretion of enzymes which are absorbed on to the style, the activity in the stomach fluid resulting from the dissolution of the style and concomitant release of enzymes (Morton, 1958a).

Cellobiase (β-glucosidase) activity was observed in all the organs examined, but was especially high in the gland of Leiblein, an organ whose apocrine secretion has been referred to before. The problem again arises as to whether a true cellobiase is responsible or whether a rather broad range β-glucosidase is acting. In a review of the subject, Pigman (1941) concludes that the evidence does not favor the concept of one enzyme responsible for the hydrolysis of all β-glucosides. He proposed that " β-glucosidase " is not a single enzyme, strictly speaking, but rather a class of closely related enzymes, which all show an ability to hydrolyze β-glucoside linkages. However, Fisher (1964), working with a partially-purified β-glucosidase from the roach, *Blaberus craniifer*, found that this presumably single enzyme was able to hydrolyze six β-glucosides including cellobiose, phenyl-β-D-glucoside, p-nitrophenyl-*p*-D-glucoside, salicin, arbutin, and gentiobiose.

The foregoing does not take into account enzymes which are active on long chain β-glucoside polymers such as cellulose and its derivatives. Evidence on this score is much more satisfactory as it has been repeatedly shown that cellulases from widely different sources attack only the polysaccharide; that cellobiose is the smallest product formed; and that cellulase and cellobiase can be separated into distinct entities, chiefly by chromatography (Pigman, 1950). From the data presented in Tables 3 and 4 it would appear safe to say that a cellobiase (or a β-glucosidase with a marked specificity for cellobiose) is present in the gland of Leiblein and that the activity observed in the stomach fluid and crystalline style has as its site of origin the apocrine secretion of the gland of Leiblein. The activity in the midgut gland is presumably endogenous and may or may not be correlated with the cellulase activity reported below.

A small amount of α-galactosidase activity was detected in the midgut gland using melibiose as substrate. Studies on yeast glycosidases indicate that α-galactosidase is a true entity, being separable from other glycosidases (Veibel, 1950). The low activity detected in the midgut gland perhaps indicates a metabolic function rather than a truely digestive one.

The β-galactosidase activity found in the gland of Leiblein and midgut gland may be due to a relatively unspecific β-glucosidase found in the organs. It is known that practically all β-glucosidase preparations are able to hydrolyze β-galactosides, although there exist β-galactosidases which can be freed of β-glucosidase activity (Veibel, 1950). Beta-glucosidase and β-galactosidase activities in *Nassarius obsoletus* can readily be interpreted as resulting from enzymes solely of the β-glucosidase type showing $\frac{1}{2}$ to 1/3 the activity with a β-galactoside as substrate. Unlike the evidence suggesting the existence of a specific cellobiase, there have been no studies reported in

which a lactase has been separable from β-galactosidase activity.

Alpha-amylase of metazoan origin is known to catalyze the hydrolysis of α-1, 4-glucosidic linkages of polysaccharides such as starch, glycogen and their derivatives. It has been further characterized as being distinct from α-glucosidases which act on smaller molecules; as having no hydrolytic activity on the α-1, 6-glucoside branch points in complex polysaccharides; and as having as its primary products larger oligosaccharides (dextrins) which are later broken down to yield maltose, isomaltose, and branched-chain products of low molecular weight (Baumann & Pigman, 1957). The amylase values shown in Table 4 are derived from somewhat indirect evidence, namely the formation of glucose. Since all preparations with presumed amylase activity also have high maltase activity, there seems no reason to doubt that an α-amylase is present which converts the starch and glycogen into disaccharides, which in turn are broken down by endogenous maltase liberating glucose.

Cellulases act on the β-glucoside linkages of complex homopolymers such as cellulose and its derivatives. It is the consensus that, as for the α-amylases, the cellulases have distinct enough properties to warrant separation from α-glucosidases (Pigman, 1950). Little significance, however, can be attached to the cellulase activity shown in Table 4, for although the first unequivocal preparation of a cellulase was derived from a gastropod mollusc (the pulmonate, *Helix*), further studies have shown that many of the reported cellulases of presumed animal origin were, in fact, due to microbial contamination (Florkin & Lozet, 1949); Stone & Morton, 1959). Although both filtration and toluene were used to remove possible bacterial activity in the tissue preparations, the resulting cellulase activity must be viewed cautiously since it is known that microbial cellulases are of the soluble extracellular type which would not be removed by filtration or added toluene. Isolation and cultivation of bacteria present in the tissues and gut of *Nassarius obsoletus* appears to be the only way to resolve the source of the enzyme.

Definitions of the terms lipase and esterase have usually been based on the chain length of the carboxylic acid. Thus, " lipase " has referred to esterases capable of attacking fatty acid esters with a long carbon chain, especially, fats, and " esterases " (or " aliesterases ") to enzymes attacking short-chain aliphatic esters. More recent classification divides fatty acid esterases into esterases acting on substrates in solution (esterases proper) and esterases (lipase-type esterases) which act predominantly on undissolved substrates (Hofstee, 1960). In the method employed in this study, a suspension of 2-naphthyl laurate was used as substrate. The principal of the determination is that lipase and esterase hydrolyze 2-naphthyl laurate to 2-naphthol and lauric acid. In the absence of a surface-active agent (taurocholate) most of the hydrolysis is due to esterase, while in the presence of taurocholate the hydrolysis is due to lipase and esterase. The difference presumably corresponds to lipase activity. As Fig. 33 indicates, there is considerable hydrolytic activity (*ca.* 800 micromoles/gram/hour) shown towards the substrate. Addition of a surface-active agent more than doubles the rate at which the substrate is hydrolyzed by the preparation, and this activation, when plotted relative to pH, indicates that most probably an esterase of the lipase-type is present along with their esterases.

Proteases are usually classified as exopeptidases or endopeptidases according to whether they act on terminal (amino or carboxy) amino acids or internal peptide linkages. From the protease activities presented in Table 5, and from the

TABLE 6. Summary of hydrolytic enzymes detectable in the digestive system of *Nassarius obsoletus* by *in vitro* methods. Preparations of high activity are italicized.

Source	Enzymatic activity	Substrate
Salivary glands	α-glucosidase	maltose
	β-glucosidase	cellobiose
Gland of Leiblein	α-glucosidase	maltose
	β-glucosidase	cellobiose
	protease	casein
Crystalline style	*α-glucosidase*	maltose
	β-glucosidase	cellobiose
	α-amylase	starch, glycogen
Stomach fluid	*α-glucosidase*	maltose
	β-glucosidase	cellobiose
	α-amylase	starch, glycogen
	(cellulase?)	carboxymethyl-cellulose
	protease	casein
Midgut glands	α-glucosidase	maltose
	β-glucosidase	cellobiose
	α-galactosidase	melibiose
	β-galactosidase	lactose
	glucosido-invertase	sucrose
	α-amylase	starch, glycogen
	(cellulase?)	carboxymethyl-cellulose
	esterase	2-naphthyl laurate
	lipase	2-naphthyl laurate
	protease	casein

method of determining protein (namely by coloration of aromatic amino acids), it would appear that the only type of protease capable of rendering soluble enough aromatic amino acid residues to give such high readings would be of the endopeptidase category. The resulting soluble protein is most probably a mixture of relatively short-chained peptides rather than a solution of amino acids. From the fact that greater activity was observed at pH 6·0 than was seen at pH 8·0, it may be tentatively assumed that the enzyme is of the trypsin type.

Table 6 summarizes the enzyme complement of the digestive organs of *Nassarius obsoletus* as revealed by this *in vitro* study.

V. ASPECTS OF DIGESTIVE PHYSIOLOGY AND BEHAVIOR

Much of the general behavior of *Nassarius obsoletus* has been discussed by Dimon (1905), Copeland (1918), Jenner (1956a, 1957 and 1958), Scheltema (1964), and Carr (1967). The following is a brief synthesis of the knowledge relating to distribution and feeding activities, drawn from the above-mentioned studies and confirmed and (in places) amplified by the present investigator.

1. *Nassarius obsoletus* is found on mud/

sand flats from a few inches above the average low tide level to approximately 10 or 12 feet below it.

2. The tidal flats on which *N. obsoletus* occurs are characteristically rich in organic material.

3. On these tidal flats, *N. obsoletus* is the numerically dominant gastropod species.

4. The distribution of these snails is not random; the snails showing, instead, a marked tendency for forming (and apparently, shifting and reforming) extensive aggregations.

5. In these aggregations, *N. obsoletus* is present in enormous numbers. Data from the Invertebrate Zoology class at the Marine Biological Laboratory at Woods Hole gave peak densities of 5860 snails per meter^2 for aggregations of adult snails at North Falmouth, Massachusetts (F.M. Fisher, personal communication). The biomass of living snail tissue at this density (at 0·5 gm living tissue/snail) equals approximately 3 kilograms/meter^2. Scheltema (1961) reports densities of 23,000/meter^2 for newly-settled larvae.

6. On the mud-sand flats, *Nassarius* is usually found moving very slowly along the surface, scooping up quantities of the substratum with its proboscis only partially extended (Fig. 35).

7. *Nassarius* will feed only when completely covered by water, or at least when there is enough water present to cover its shell aperture.

8. *N. obsoletus* in nature is primarily a deposit-feeder. The stomach contents of snails examined in the field uniformly consisted of great quantities of sand, mud, and organic detritus.

9. *Nassarius* in nature has been observed to feed actively on the larger algae (such as *Ulva*) and in the laboratory it will graze on algal scum covering the walls of aquaria.

10. In nature and in the laboratory, snails show a marked preference for the flesh of dead animals. *Nassarius* has been observed to eat the following (dead) animals: *Mya*, *Mytilus*, *Modiolus*, *Nassarius*, *Littorina*, *Nereis*, *Squilla*, hermit crabs, and frozen shrimp (*Penaeus*). In addition, Dimon (1905) reports observing a living nereid being devoured by a cluster of *Nassarius* in the field, but this was apparently an exceptional instance.

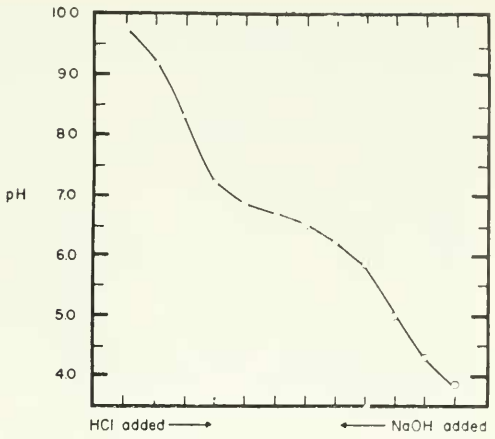


FIG. 34. Titration curve of crystalline styles in solution.

11. *N. obsoletus* exhibits a distinct behavioral response to the presence of decaying meat. In order of occurrence, the following events take place: (*a*) Initial detection of soluble diffusing substances from the meat leads to an overall increased activity. Animals which are partially or completely buried extend their siphons and, after a short interval, come rapidly to the surface of the substratum. (*b*) This increase in activity is immediately followed by relatively rapid forward locomotion accompanied by a constant sweeping of the siphon from side to side in approximately 120° arc in front of the snails. (*c*) After a brief period of randomly-directed forward locomotion, the snails orient themselves against the direction of the current flow (rheotaxis) and move upstream. (*d*) The snails continue

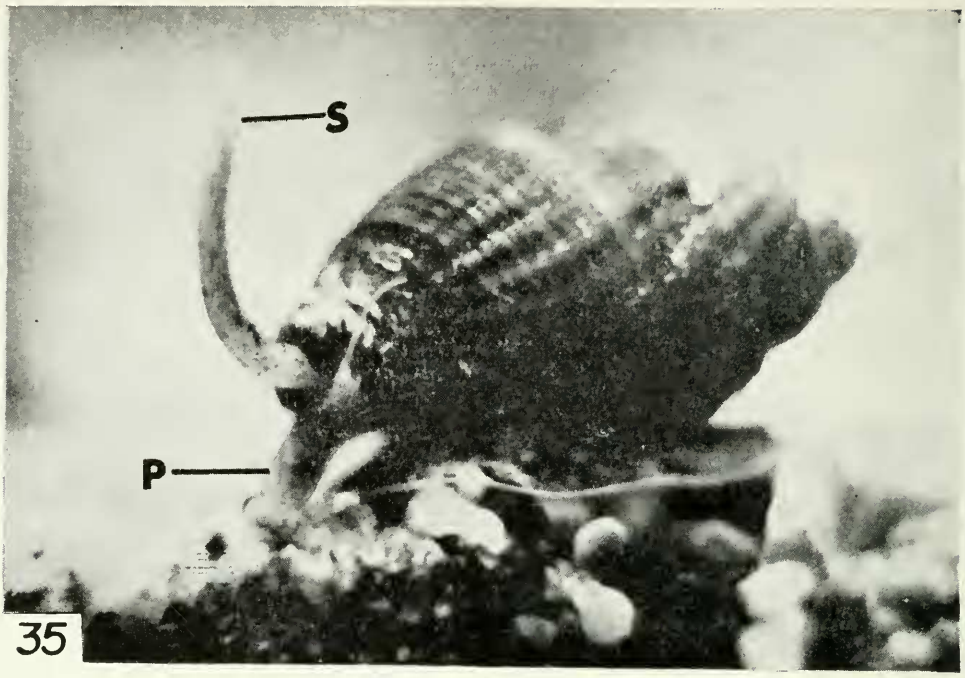


FIG. 35. *Nassarius obsoletus* with proboscis extended as far as the substratum. This is its normal position when the animal is feeding on surface detritus.

movement upstream with "searching" movements of their siphon and, as the meat is neared, the proboscis is extended and radular action begins (Fig. 36). (*e*) Upon reaching the meat, the proboscis is applied to the surface and, by radular action, the proboscis literally bores a hole deep into the food mass (see also Carr, 1967a and 1967b).

12. That the initial response to meat is chemical rather than visual is easily shown by the following facts: (*a*) In nature, animals which are close by, but upstream from, a decaying piece of meat do not become characteristically active or exhibit any of the behavioral traits associated with the detection of meat (as in 11, above). On the other hand, animals which are much farther away, but downstream, from the same piece of meat *do* become active and go through the searching movements, eventually reaching and eating the meat. (*b*) In the laboratory, a single drop of meat juice introducted into the aquarium is sufficient to elicit the easily-observed responsive activities discussed under 11 (a-b), above.

13. Clear evidence for a rheotaxis is provided by the behavior of animals in nature preparatory to feeding [as in 11 (*c*), above] and by the following observations of animals under laboratory conditions: (*a*) If a drop of meat juice is added to a battery jar containing snails and the water stirred so as to give a unidirectional current (clockwise, for example), the animals become active and, after a few moments of randomly-directed locomotion, move with searching movements against the direction of the current (counterclockwise in this case). By reversing the direction of the current, the animals will turn 180° in their path and more as before against the current. (*b*) If

a piece of meat is dropped into the middle of an aquarium in which the water has been allowed to become still, the snails become active, but mill about with no uniform directional heading. Those that do perchance contact the meat stop and begin feeding, and thereby many snails eventually find the food, but by the mechanism of klinokinesis (Fraenkel & Gunn, 1940) rather than by a directed taxis.

The time of retention of food in the digestive tract has been used as an indication of the efficiency of the digestive process (Prosser & Brown, 1961). In *N. obsoletus* just taken from its natural habitat and isolated in aquaria, the gut is completely emptied of sand and mud in approximately 12 hours. Observations on previously-starved animals fed on frozen shrimp indicate that the passage of this type of food is completed more rapidly, often in as short a period as 4 hours. Whether or not the observed feeding times can be directly correlated with the "efficiency of the digestive process" remains an unanswered question. Nevertheless, it would not be too surprising if the digestion of organic material within a matrix of sand and mud particles would be less efficient than digestion of a concentrated "pure" food form such as animal tissues. What physiological mechanisms may be involved in regulating the speed of food flow through the gut can only be guessed.

As Jenner (1956b) has pointed out, the mechanism of primary importance in propulsion of material through the digestive tract is one of peristaltic contraction of the walls of the alimentary canal. As has been shown (part 1, above), the musculature surrounding the various portions of the gut is well-developed, and thus the anatomical basis for such peristaltic movements is clearly established.

The formation of fecal material, its consolidation and elimination, are thought to have been at least partially responsible for many of the evolutionary specializations found in the Gastropoda (for example: the extensive elaboration of mucous glands in the intestine; the formation of food/fecal strings; the shift of the anus to the right side of the pallial cavity; the great reliance on, and success of, the pectinibranch ctenidium; the shift in pallial water currents from ventro-dorsal to lateral; and so on). In light of such theoretically important considerations, it is of interest to note the conditions which obtain in *Nassarius obsoletus*. Although mucous goblet cells abound in the intestinal region, no discrete consolidated fecal pellets, such as are known from microphagous hervibores, are formed by *N. obsoletus*. If animals are taken from the field and placed in clean seawater-filled finger bowls, defecation can be observed and fecal products examined. The great bulk of expelled material consists of sand particles which have mucus adhering to them. The mucus has insufficient binding capability, however, to hold the heavy sand grains together in a fused mass. Presumably the size and weight of the particles cause them to settle rapidly out of the mantle cavity and thus prevent them from interfering with the ctenidium and the respiratory currents. Lighter and more finely divided material is held together somewhat better than the sand grains, but the compactness of consolidation does not approach that found in forms subsisting solely on a diet of minutely-divided particulate material.

Characteristic of the molluscan stomach is the presence of numerous ciliated folds and ridge systems which act as particle "sorting fields". These are particularly well-developed in the lamellibranch Bivalvia and in those Archaeogastropoda and Mesogastropoda which are of the continuously-grazing microherbivore type. The food strings and crystalline styles of lamellibranchs and style-bearing proso-

branchs are likewise propelled by extensively ciliated surfaces. In *N. obsoletus*, the entire alimentary canal with the exception of the caecum is lined by ciliated epithelium as shown in part I. The stomach, however, is simplified with regard to sorting fields in comparison to most of the lower gastropods. It does, however, retain vestiges of organized ciliated fields which are often absent in the more specialized Neogastropoda. The following ciliary currents were determined by the use of finely divided particulate material such as carmine and carborundum.

Issuing from the esophageal opening (Fig. 18, OPE), a relatively weak current proceeds posteriorly for a very short distance and then terminates abruptly at the anterior edge of the caecal folds. No ciliary activity could be observed along the folded walls of the caecum itself. To the left of the esophageal opening a series of currents run along the small transverse folds converging on to the smooth saddle-shaped area (SSA). Although this region of transverse folds most closely resembles a sorting field of the type found in lamellibranchs and lower gastropods, there is no sign of the characteristic separation of particles by size or of the presence of two currents perpendicular to each other to effect such a separation.

To the right of the esophageal opening are found currents issuing from the openings of the migdut glands and a current directed away from the ventral midline across the large area of smooth epithelium adjacent to the gastric shield. Within the sulcus forming the posterior boundary of the typhlosoles are found strong currents directed medially towards the ventral intestinal groove. A strong current continues along the floor of this groove carrying particles entrapped in mucus anteriorly toward the intestine. Strong ciliary activity is found on both typhlosoles: a posteriorly-directed current along the face of the minor typhlosole (MiT) presumably forces the crystalline style backward against the gastric shield, while ciliary activity directed medially along the surface of the major typhlosole (MaT) causes the style to rotate in a clockwise direction when viewed from the rear. Currents on the sides of the typhlosoles are directed ventrally and serve to carry particles into the anteriorly flowing currents of the intestinal groove.

In *Nassarius obsoletus*, therefore, there is no evidence that the stomach accomplishes any particle separation through the mechanism of ciliary sorting fields.

Perhaps the most notable feature of the stomach of *Nassarius obsoletus* is the presence of a crystalline style. Functionally, the crystalline styles of lamellibranchs and lower gastropods are thought to act as: (1) repositories for digestive enzymes: (2) " capstans " which aid in drawing mucus food strings into the stomach; and (3) buffer sources to maintain the pH of the stomach fluid (Morton, 1952 and 1960). It is of interest to note how the style of *N. obsoletus* compares with styles found elsewhere in the Mollusca with regard to these functions.

It has been clearly demonstrated that the style of *Nassarius obsoletus* does contain hydrolytic enzymes (part III, above). It is unlikely, however, that the style of these animals in nature acts as a capstan to any significant extent, since, as has been discussed above, the bulk of ingested material is sand and coarse mud (coated, but not tightly bound, by mucus) which is passed along the alimentary canal by muscular peristalsis.

In an effort to determine whether or not the style of *Nassarius obsoletus* has any buffering capability, 10 styles were allowed to dissolve in 10·0 ml of glass-distilled water. The resulting solution was titrated with 0·01 N HCl and 0·01 N NaOH and the pH determined with a Sargent model PB pH meter. The titra-

tion curve is given in Fig. 34. It shows buffering action between pH 5·8 and 7·2, the midpoint being at pH 6·5. This agrees well with values for the stomach fluid of pH 6·0–6·5 obtained by the use of indicators (bromthymol blue and bromcresol purple).

The style of *Nassarius obsoletus*, therefore, apparently does have a buffering function in addition to the enzymatic one discussed above.

VI. GENERAL DISCUSSION

Studies on the functional morphology of molluscs by Atkins, Fretter, Graham, Morton, and Yonge, among others (reviewed by Morton, 1958a; Fretter & Graham, 1962; Wilbur & Yonge, 1964; Owen, 1966; and Hyman, 1967), offer convincing evidence that the first molluscs most probably all fed on small particles. These particles were non-selectively scraped up from the substratum by the radula, bound by mucous secretions into a "food string", transported along the alimentary canal by ciliary activity, and eventually subjected to phagocytosis and intracellular digestion within the blind tubules of the midgut gland. Such dependence on the intracellular mode of digestion imposed the requirement that the food particles presented to the digestive cells be within certain size limits to allow for phagocytosis. Among the earliest evolutionary features to appear in molluscs, therefore, were mechanisms designed to grade and sort particles according to size and to transport the sorted particles to their proper destinations within the digestive tract. The extensive use of mucous secretions to bind the particulate food material together for transport through the alimentary canal led to the production, within the stomach, of a mucoprotein rod, the forerunner of the crystalline style, or protostyle. This rod gained increased functional significance as it assumed the mechanical burden of drawing the mucus food-string into the stomach, as it became a repository for extracellular amylases, and as it added a buffering effect to maintain the pH of the stomach.

The lamellibranch bivalves adopted the habit of feeding on particles suspended in the surrounding water and thus avoided the larger particulate material which made up the bulk of the ingested matter of deposit feeders. Further refinement of food selection was achieved by the use of ciliary sorting fields on the labial palps and within the stomach itself. Digestion in this group has *presumably* remained for the most part intracellular, although a partial breakdown does occur extracellularly of material, such as polysaccharide, the digestion of which is comparatively difficult.

The gastropods, with notable exceptions, retained use of the radular apparatus to scrape up food material from the substratum in a non-selective manner. Early dietary specialization led some gastropods to become microphagous herbivores, feeding primarily on algal fragments rasped from rocks and other hard surfaces. Sorting by size of particle was accomplished almost solely by means of ciliarly sorting fields within the stomach —these functioning similarly to those found in the Bivalvia.

Among living prosobranchs, some of the Archaeogastropoda and Mesogastropoda retain the habit of microphagous herbivory although the evolutionary trend has been for gastropods to adopt macroherbivorous or carnivorous habits. The mesogastropod microherbivores retain possession of ciliary sorting fields within the stomach, and certain entire superfamilies (Rissoacea, Cerithiacea, and Calyptraeacea) are characterized by the possession of a crystalline style. Here, as in the lamellibranchs, the primary mode of digestion is intracellular, with

partial extracellular digestion taking place by means of crystalline style enzymes.

The rachiglossan Neogastropoda (including the superfamilies Buccinacea, Muricacea, and Volutacea) are characteristically carnivorous. The modifications which have occurred to equip such snails for a diet of animal flesh include: (1) development of the rachiglossan radula, possessing three sharp-cusped teeth per row, which is extremely well-suited for tearing bits of flesh from solid animal tissue; (2) size increase and elaboration of the proboscis which allows penetration of the feeding apparatus deep into animal tissues and into relatively inaccessible places such as between bivalve shells and into tunicate tests; (3) extension of the mantle tissue into a long movable canal (the siphon) which allows delicately-controlled intake of the surrounding water which is then directed over (4) a well-developed bipectinate osphradium which is employed as a chemosensory organ for the detection of food; (5) development of a valvular device in the esophagus (the valve of Leiblein) which allows protrusion and elongation of the proboscis without regurgitation of food material; (6) essentially complete conversion to extracellular digestion; (7) specialization of glands (such as the salivary glands, gland of Leiblein, and midgut gland) to produce extracellular enzymes; (8) simplification of the stomach into a bag where enzymes and food are mixed and digestion occurs, and from which soluble material passes into the ducts of the midgut gland for absorption; (9) great reduction or complete loss of ciliary sorting fields, since there is no longer the requirement for separation of particles from one another according to size; (10) loss of a crystalline style, since the proteinaceous style presumably would be digested by the extracellular proteases of strictly carnivorous forms; and (11) great reduction or more often complete loss of the gastric shield, since with the crystalline style absent, there no longer is abrasion between a style head and the lining epithelium.

The Buccinacea amongst the Neogastropoda are known to be the least specialized of the carnivorous Rachiglossa. Within the Buccinacea, members of the family Buccinidae frequently eat living flesh, while the family Nassariidae characteristically feed on dead or decaying animal matter.

The anatomy of the *Nassarius* species studied agrees in almost every detail with the characteristics listed above associated with assumption of a carnivorous existence. Thus, the presence of the rachiglossan radula, the extremely long and protrusible proboscis, the long siphon and bipectinate osphradium, the well-developed valve of Leiblein, salivary glands, and gland of Leiblein, the simplification of stomach structure, the absence of efficient sorting ciliate regions, and the reduced gastric shield—all bespeak the typical carnivorous rachiglossan structure.

Likewise, almost all of the species of *Nassarius* are described as being carnivorous, subsisting on a diet of dead and decaying animal flesh (Blegvad, quoted in Yonge, 1954; Graham, 1955; Morton, 1958a; Fretter & Graham, 1962; and Martoja, 1964).

In addition to exhibiting the anatomical characteristics listed above, however, *Nassarius obsoletus* also possesses a crystalline style, and in apparent contrast to the other *Nassarius* species, *N. obsoletus* is clearly a deposit feeder. There can be very little doubt that in its natural habitat *N. obsoletus* receives almost all of its nutrition from the organic debris found within the mud and silt of the intertidal flats. This organic debris to the greatest extent consists of living unicellular algae, algal degradation products, and attendant micro-organisms.

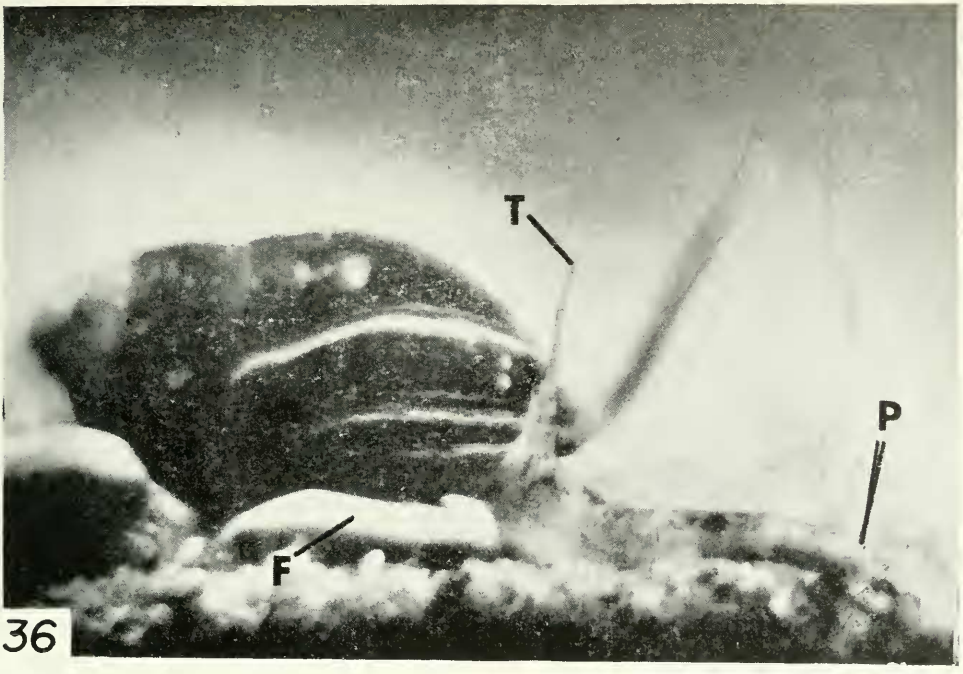


FIG. 36. *Nassarius obsoletus* with its proboscis greatly (but not completely) extended. This animal was preparing to feed on a piece of dead meat (out of camera range).

From Newell's (1964) study, it is apparent that certain deposit-feeding molluscs actually digest only the protein derived from the microbial coating on the silt and organic debris. In the two species of deposit-feeders studied (*Hydrobia ulvae* and *Macoma balthica*), the amount of ingested organic carbon (presumably derived from living unicellular algae and algal degradation products) was almost totally recoverable in the feces, while the organic nitrogen ingested (derived mainly from bacterial synthesis) was retained (and presumably metabolized) by the molluscs. Although experiments to determine carbon and nitrogen ingestion/egestion ratios have not yet been done for *N. obsoletus*, the author concurs with Scheltema (1964) that most probably the main nutritional component of the ingested material is the microfloral carbohydrate and not the bacterial protein. The presence of crystalline styles and concommitant absence of extracellular proteases within the stomachs of *N. obsoletus* on the mud flats argues strongly for this conclusion.

From the data presented in this study, we may attempt to describe *Nassarius obsoletus*, although anatomically a carnivore, is able to handle a herbivorous (or more strictly an omnivorous) diet.

The present findings all point to the fact that intracellular digestion, either by migrating amoebocytes, or by cells of the midgut gland, does not occur to any significant extent. The evidence bearing on this point includes the observations that: (1) *Nassarius obsoletus* clearly lacks any efficient mechanism (ciliary or otherwise) to sort and separate particles according to size. Such a mechanism is obviously a prime requisite in view of the extreme variation in size range of the

ingested material. (2) There is no uptake by the midgut gland cells or amoebocytes of finely particulate material such as carmine or carborundum, nor is there any histological evidence of food vacuole formation in the midgut gland cells. (3) Acid phosphatase activity in the midgut gland cells is confined to the lumenal border rather than having particulate localization in the more basal cytoplasm (which would be expected if phagocytosis, and hence lysosomal activity, occurred). (4) No histological evidence was observed of amoebocytes being within the lumen of the digestive tract or between the cells of the lining epithelium, nor did amoebocytes within the midgut gland haemocoel show a positive reaction in any of the histochemical procedures employed for the demonstration of hydrolytic enzymes.

On the other hand, data from the *in vitro* enzyme determinations reveal the presence of a variety of enzymes within the stomach lumen and in extracts of the crystalline style, thus strongly suggesting that extracellular digestion does indeed take place. The crystalline style itself contains several carbohydrate-splitting enzymes including α-glucosidase, β-glucosidase, and polysaccharases capable of hydrolyzing starch and glycogen. The stomach fluid likewise contains enzymes like those of the crystalline style (and most probably derived from it) and, in addition, it has definite traces of glucosido-invertase, α-galactosidase, β-galactosidase, and cellulase activity. These findings (along with the histochemical and/or *in vitro* demonstration of esterase, lipase, and β-glucuronidase activity within the midgut gland) offer strong evidence that the digestive system of *Nassarius obsoletus* has sufficient hydrolytic enzymes to digest and ultimately metabolize the algal constituents (such as structural polysaccharides and various esters and polymers of galactose and uronic acids) which form the greatest proportion of its ingested food material (Fox, 1950; Black, 1954).

Extracellular protease activity (Table 5) was found in the stomach fluid of certain animals just taken from the field, the styles being absent from these animals. This fact, and the observation that snails which were maintained in the laboratory exclusively on a diet of meat invariably lacked styles and gastric shields, can best be explained following Yonge's (1930) reasoning that a proteinaceous crystalline style cannot co-exist with extracellular proteolytic enzymes without itself being subject to dissolution by enzymatic action. The presence of a style in a snail can be taken as clear evidence for the absence of extracellular proteases. Animals feeding on mudflats unquestionably ingest some animal tissues and micro-organisms as a matter of course; the presence of a style indicates, however, that they cannot be digesting these materials extracellularly. The ingestion of large quantities of animal flesh, such as occurs regularly during laboratory maintainance, or sporadically in nature, apparently elicits release of extracellular proteases which digest meat (as well as style) protein. The intriguing questions which arise here involve: (1) the apparent reciprocal relationship between the presence of a style versus the presence of extracellular proteases in the lumen of the stomach; and (2) the influence (control?) exercised over these by the type of food ingested.

The site of secretion of the enzymes found in the stomach fluid and crystalline style is not known with certainty. It seems probable, however, that the gland of Leiblein, midgut gland, and perhaps salivary gland are chiefly responsible for such enzyme production. In particular, the high tissue activities of protease, α-glucosidase, and β-galactosidase found in the gland of Leiblein suggest that these enzymes are derived primarily from this source. Similarly, it seems not improb-

able that the midgut gland is the primary source of glucosido-invertase, α-galactosidase, and the polysaccharases which act on starch and glycogen. As stated before, the origin of the cellulase activity is very much in doubt—a microbial origin, however, seems not unlikely.

The site of uptake of the digested food awaits final clarification from further studies. The presence in *Nassarius obsoletus* of microvilli along the lumenal border of the columnar cells in the midgut gland agrees with the findings of Summer (1966) who, by electron microscopy, demonstrated the presence of microvillar brush borders in the midgut gland cells of the pulmonate *Helix*. Sumner also showed the presence of pinocytotic vesicles and channels extending into the cytoplasm of the midgut gland cells. Terrestrial pulmonates such as *Helix* are macroherbivores in which extracellular digestion occurs in a thin-walled stomach and absorption of the soluble food material occurs in the midgut gland. It is not unlikely, therefore, that the midgut gland of *N. obsoletus* has a similar absorptive function.

The presence of phosphatases along the lumenal borders of the midgut gland cells, as has been mentioned previously, is also indicative of metabolically active cell surfaces. There has been little evidence until recently that uptake of soluble digestive products in non-cephalopod molluscs occurs elsewhere than in the midgut gland tubules. Recent studies by Greer & Lawrence (1966) and Lawrence & Lawrence (1966) have shown, however, that isolated intestinal segments of the polyplacophoran *Cryptochiton stelleri* are able to actively transport basic and neutral amino acids and the monosaccharides D-glucose, 3-0-methyl glucose, and D-galactose. The results of these studies suggests that, for *C. stelleri* at least, the gut is of greater importance than the midgut gland in the uptake of soluble digestive products. This may well prove to be true for many other molluscs, including *Nassarius obsoletus*.

Following conventional descriptions of dietary preference and digestive capability, one must classify *Nassarius obsoletus* as an omnivore. The omnivory practiced by *N. obsoletus*, however, is significantly different from that found in most other animals. Although it is capable of feeding on, and utilizing, both plant and animal materials, *N. obsoletus* apparently "commits" itself to one or the other, rather than feeding on and digesting both simultaneously. The "commitment" is to some degree forced upon it by circumstance. The presence of a style in typical mud flat snails indicates that no proteases are normally present in the lumen of the gut and hence even though some animal material is undoubtedly taken in, there are no extracellular enzymes present to digest it. Such an animal is functionally a total herbivore. On the other hand, when a piece of carrion is present on the mud flats, the snail shows a strong preference for this and will attack it to the exclusion of its normal fare. At such times both proteases and carbohydrases are present in the stomach, but due to the strong behavioral response, the snail has ensured that it will eat a meal of essentially pure meat. During this time the animal is functioning solely as a carnivore. It seems more accurate, therefore, to classify the snail as a *facultative herbivore/carnivore* rather than as an omnivore.

The adaptive value of such a digestive mechanism in a mud flat snail seems reasonably clear-cut. It permits utilization of the algal debris deposited at each receeding tide and yet allows for the utilization of the occasional bit of carrion washed up on the flats. The origin of such a habit is more obscure. Presumably the ancestral stock could not compete in other regions with the more

efficient mesogastropod microherbivorous grazers such as *Littorina* or with the more specialized stenoglossan carnivores such as the whelks and drills. Its unique digestive mechanism has permitted evolutionary success in the mud flat habitat.

In conclusion, the data show that *Nassarius obsoletus*, although possessing the many structural modifications associated with a carnivorous mode of feeding and digestion, nevertheless has been able to utilize a primarily herbivorous diet. From the anatomical evidence alone, this appears to be a secondary adaptation derived from a principally carnivorous ancestry. There is nothing in the structure of *N. obsoletus* to suggest that it is an intermediate form of a basically herbivorous line which is in the process of "becoming" carnivorous. Physiologically, the presence of secreted hydrolytic enzymes and a functional crystalline style permits extracellular digestion of algal components—a situation necessitated by the absence of mechanisms for sorting particles according to size (a prerequisite for any significant amount of phagocytosis and intracellular digestion). The crystalline style of *Nassarius obsoletus*, apparently absent in the other *Nassarius* species, is likely a neomorphic addition. There is no evidence that any of the Buccinacea have evolved directly from any of the style-bearing mesogastropod groups, and furthermore it is thought not unlikely that styles have been evolved several times within the Mollusca (Robson, 1922; Yonge, 1932; and Morton, 1960).

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LITERATURE CITED

ADAMS, C. W. M., 1957, A p-dimethylaminobenzaldehyde nitrite method for the histochemical demonstration of tryptophane and related compounds. *J. clin. Path.*, 10: 56–62.

BARKA, T. & ANDERSON, P. J., 1963, *Histochemistry. Theory, Practice and Bibliography.* Harper & Row, New York, 660 p.

BATCHELDER, C. H., 1915, Migration of *Ilyanassa obsoleta*, *Litorina litorea*, and *Litorina rudis*. *Nautilus*, 29: 43–46.

BAUMANN, H. & PIGMAN, W. W., 1957, Naturally occurring glycosides and glycosidases. In: *The Carbohydrates*, (W. Pigman, ed.) Academic Press, New York, p 536–600.

BAYLOR, E. R., 1958, The responses of snails to polarized light. *J. exp. Biol.*, 36: 369–376.

BERG, W. E. & KATO, Y., 1959, Localization of polynucleotides in the egg of *Ilyanassa*. *Acta. Embr. Morph. Exp.*, 2: 227–233.

BILLETT, F. & McGEE-RUSSELL, S. M., 1955, The histochemical localization of β-glucuronidase in the digestive gland of the Roman Snail (*Helix pomatia*). *Quart. J. micros. Sci.*, 96: 35–48.

BLACK, W. A. P., 1954, Constituents of the marine algae. *Ann. Rep. Chem. Soc.*, (*Biochem.*), 50: 322–335.

BROCK, F., 1936, Suche, Aufnahme und enzymatische Spaltung der Nahrung durch die Wellhornschnecke *Buccinum undatum*. *Zoologica*, 34: 1–136.

BROWN, F. A., WEBB, H. M. & BRETT, W. J., 1959, Exogenous timing of solar and lunar periodism in metabolism of the mud snail *Ilyanassa* (*Nassarius*) *obsoletus* in the laboratory. *Gamma J. Med. Sci.*, 8: 233–242.

BROWN, F. A., BENNETT, M. F. & WEBB,

H. M., 1960, A magnetic compass response of an organism. *Biol. Bull.*, 119: 65–74.

BURSTONE, M. S., 1955, An evaluation of histochemical methods for protein groups. *J. Histochem. Cytochem.*, 3: 32–49.

CARR, W. E. S., 1967a, Chemoreception in the mud snail, *Nassarius obsoletus*. I. Properties of stimulatory substances extracted from shrimp. *Biol. Bull.*, 133: 90–105.

CARR, W. E. S., 1967b, Chemoreception in the mud snail, *Nassarius obsoletus*. II. Identification of stimulatory substances. *Biol. Bull.*, 133: 106–127.

CATHER, J. N., 1959, The effects of x-radiation on the early cleavage stages of the snail, *Ilyanassa obsoleta*. *Rad. Res.*, 11: 720–731.

CATHER, J. N., 1963, A time schedule of the meiotic and early mitotic stages of *Ilyanassa*. *Caryologia*, 16: 663–670.

CATHER, J. N., 1967, Cellular interactions in the development of the shell gland of the gastropod, *Ilyanassa*. *J. exp. Zool.*, 166: 205–224.

CAVANAUGH, G. M., 1956, *Formulae and Methods*. IV ed., Marine Biol. Lab. Publ., Woods Hole.

CLEMENT, A. C., 1952, Experimental studies on the germinal localization in *Ilyanassa*. I. The role of the polar lobe in the determination of the cleavage pattern and its influence on later development. *J. exp. Zool.*, 121: 593–626.

CLEMENT, A. C., 1956, Experimental studies on the germinal localization in *Ilyanassa*. II. The development of isolated blastomeres. *J. exp. Zool.*, 132: 427–446.

CLEMENT, A. C., 1960, Development of the *Ilyanassa* embryo after removal of the mesentoblast cell. *Biol. Bull.*, 119: 310.

CLEMENT, A. C., 1962, Development of *Ilyanassa* following removal of the D macromere at successive cleavage stages. *J. exp. Zool.*, 149: 193–216.

CLEMENT, A. C. & LEHMANN, F. E., 1956a, Uber das Verteilungsmuster von Mitochondrien und Lipoidtropfen wahrend der Furchung des Eies von *Ilyanassa obsoleta*. *Die Naturwiss.*, 20: 478–479.

CLEMENTS, A. C. & LEHMANN, F. E., 1956b, The distribution of mitochondria and lipid droplets during early cleavage in *Ilyanassa obsoleta*. *Biol. Bull.*, 111: 300.

CLEMENT, A. C. & TYLER, A., 1967, Protein-synthesizing activity of the anucleate polar lobe of the mud snail *Ilyanassa obsoleta*. *Sci.*, 158: 1457–1458.

COLLIER, J. R., 1960, The localization of some phosphorous compounds in the egg of *Ilyanassa obsoleta*. *Exp. Cell Res.*, 21: 548–555.

COLLIER, J. R., 1961, The effect of removing the polar lobe on the protein synthesis of the embryo of *Ilyanassa obsoleta*. *Acta Embr. Morph. Exp.*, 4: 70–76.

COPELAND, M., 1918, The olfactory reactions and organs of the marine snails *Alectrion obsoleta* and *Busycon canaliculatum*. *J. exp. Zool.*, 25: 177–228.

CRAMPTON, H. E., 1896, Experimental studies on gasteropod development. *Arch. Entw. mech.*, 3: 1–19.

DAHLQVIST, A., 1961, Determination of maltase and isomaltase activities with a glucose-oxidase reagent. *Biochem. J.*, 80: 547–551.

DAN, K. & DAN, J. C., 1942, Behavior of the cell surface during cleavage. IV. Polar lobe formation and cleavage of the eggs of *Ilyanassa obsoleta* Say. *Cytologia*, 12: 246–261.

de DUVE, C., 1959, Lysosomes, a new group of cytoplasmic particles. *In*: "Subcellular Particles," *Am. physiol. Soc.*, Wash., D.C. (T. Hayashi, ed.) p 128–159.

de DUVE, C. & WATTIAUX, R., 1966, Functions of lysosomes. *Ann. Rev. Physiol.*, 28: 435–492.

DEMPSEY, E. W. & SINGER, M., 1946, Observations on the chemical cytology of the thyroid gland at different functional stages. *Endocrinology*, 38: 270–295.

DIMON, A. C., 1905, The mud snail: *Nassa obsoleta*. *Cold Spring Harbor Monogr.*, 5: 1–48.

DODGSON, K., LEWIS, J., & SPENCER, B., 1953, Studies on sulphatases. 3. The arylsulphatase and β-glucuronidase of marine molluscs. *Biochem. J.*, 55: 253–259.

FISHER, F. M., 1964, The properties and specificity of a β-glucosidase from *Blaberus craniifer*. *Biol. Bull.*, 126: 220–234.

FLORKIN, M. & LOZET, F., 1949, Origine bacterienne de la cellulase du contenu intestinal de l'Escargot. *Arch. Int. Physiol.*, 57: 201–207.

FOX, D. L., 1950, Comparative metabolism of organic detritus by inshore animals. *Ecology*, 31: 100–108.

FRAENKEL, G. S. & GUNN, D. L, 1940, *The Orientation of Animals*. Oxford Univ. Press, London 376 p.

FRETTER, V. & GRAHAM, A., 1962, *British Prosobranch Molluscs*. The Ray Society. London, 755 p.

FRUTON, J. S. & SIMMONDS, S., 1958, *General Biochemistry*. 2nd ed. John Wiley, New York, 1077 p.

GOMORI, G., 1950, An improved histochemical technic for acid phosphatase. *Stain Tech.*, 25: 81–85.

GOMORI, G., 1952, *Microscopic Histochemistry*. Univ. of Chicago Press, Chicago, 273 p.

GOTTSCHALK, A., 1950, α D-Glucosidases. *In*: *The Enzymes*, 1st ed. (J. B. Summer & K. Myrback, eds.) Academic Press, New York, p 551–582.

GRAHAM, A., 1939, On the structure of the alimentary canal of style-bearing prosobranchs *Proc. zool. Soc. Lond.*, 109: 75–112.

GRAHAM, A., 1941, The oesophagus of stenoglossan prosobranchs. *Proc. Roy. Soc. Edin.*, 61: 1–23.

GRAHAM, A., 1949, The molluscan stomach. *Trans. Roy. Soc. Edin.*, 61: 737–778.

GRAHAM, A., 1955, Molluscan diets, *Proc. malac. Soc. Lond.*, 31: 144–159.

GREER, M. L. & LAWRENCE, A. L., 1966, Active transport of amino acids by a marine invertebrate. *Amer. Zool.*, 6: 318–319.

HALE, C. W., 1946, Histochemical demonstration of acid mucopolysaccharides in animal tissues. *Nature*, Lond., 157: 802.

HESS, R. & PEARSE, A. G. E., 1958, The histochemistry of indoxylesterase of rat kidney with special reference to its cathepsin-like activity. *British J. exp. Path.*, 39: 292–299.

HIRSCH, G. C., 1915, Ernahrungsbiologie fleischfressender Gastropoden (*Murex*, *Natica*, *Pterotrachea*, *Pleurobranchaea*, *Tritonium*). I Teil. Makroskopischer Bau, Nahrungsaufnahme, Verdauung, Sekretion. *Zool. Jb.*, (Abt. Physiol.), 35: 357–504.

HOFSTEE, B. H. J., 1960, Fatty acid esterases of low eserine sensitivity and related enzymes. *In*: *The Enzymes*, 2nd ed. (P. Boyer, H. Lardy and K. Myrback, eds.) Academic Press, N.Y., p 485–498.

HOLT, S. J., 1958, Indigogenic staining methods for esterases. *In*: *General Cytochemical Method*, Vol. 1 (J. F. Danielli, ed.) Academic Press, N.Y. p 375–398.

HOLT, S. J. & WITHERS, R. F. J., 1952, Cytochemical localization of esterases using indoxyl derivatives. *Nature*, Lond., 170: 1012–1014.

HYMAN, L. H., 1967, *The invertebrates*, Vol. VI, McGraw Hill, New York, 792 p.

JENNER, C. E., 1956a, A striking behavioral change leading to the formation of extensive aggregations in a population of *Nassarius obsoletus*. *Biol. Bull.*, 111: 291.

JENNER, C. E., 1956b, The occurrence of the crystalline style in the mud snail *Nassarius obsoletus*. *Biol. Bull.*, 111: 304.

JENNER, C. E., 1957, Schooling behavior in mud snails in Barnstable Harbor leading to the formation of massive aggregations at the completion of sexual reproduction. *Biol. Bull.*, 113: 328–329.

JENNER, C. E., 1958, An attempted analysis of schooling behavior in the marine snail, *Nassarius obsoletus*. *Biol. Bull.*, 115: 337–338.

JENNER, C. E. & CHAMBERLAIN, N. A., 1955, Seasonal resorption and restoration of the copulatory organ in the mud snail *Nassa obsoleta*. *Biol. Bull.*, 109: 347.

LAWRENCE, A. L. & LAWRENCE, D. C., 1966, Active transport of monosaccharides by the intestine of the chiton, *Cryptochiton stelleri*. *Amer. Zool.*, 6: 319.

LILLIE, R. D., 1965, *Histopathologic technic and practical histochemistry*. 3rd ed. The Blakiston Co., Inc., New York, 715 p.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J., 1951, Protein measurement with the folin phenol reagent. *J. biol. Chem.*, 193: 265–275.

LUFT, J. H., 1961, Improvement in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.*, 9: 409–414.

MANSOUR-BEK, J. J., 1934, Uber die Proteolytischen Enzyme von *Murex anguliferus* Lam. *Z. Vergl. Physiol.*, 20: 343–369.

MARTIN, W. E., 1938, Studies on trematodes of Woods Hole: I. The life cycle of *Lepocreadium setiferoides* (Miller and Northup), Allocreadiidae, and the description of *Cercaria cumingiae*. *Biol. Bull.*, 75: 463–474.

MARTIN, W. E., 1939, Studies on the trematodes of Woods Hole: II. The life cycle of *Stephanostomum tenue*. *Biol. Bull.*, 77: 65–73.

MARTOJA, M., 1964, Contribution a l'etude de l'appareil digestif et de la digestion chez les gasteropodes carnivores de la famille des Nassarides. *Cellule*, 64: 237–334.

McGEE-RUSSELL, S. M., 1958, Histochemical methods for calcium. *J. Histochem.*, 6: 22–42.

MORGAN, T. H., 1933, The formation of the antipolar lobe in *Ilyanassa*. *J. exp. Zool.*, 64: 443–467.

MORTON, J. E., 1952, The role of the crystalline style. *Proc. malacol. Soc. Lond.*, 29: 85–92.

MORTON, J. E., 1958a, *Molluscs*. Hutchinson, London, 232 p.

MORTON, J. E., 1958b, The habits of *Cyclope neritea*, a stylebearing stenoglossan gastropod. *Proc. malac. Soc. Lond.*, 34: 96–106.

MORTON, J. E., 1960, Gut functions in ciliary feeders, *Biol. Revs.*, 35: 92–140.

MOWRY, R. W., 1963, The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins, with revised directions for the colloidal iron stain, the use of Alcian Blue 8GX and their combinations with the periodic acid

Schiff reaction. *Ann. N.Y. Acad. Sci.*, 106: 402–423.

MYRBACK, K., 1960, Invertases. *In*: *The Enzymes*, 2nd ed. (P. Boyer, H. Lardy & K. Myrback, eds.) Academic Press, New York, p 379–395.

NACHLAS, M. M., CRAWFORD, D. T. & SELIGMAN, A. M., 1957, The histochemical demonstration of leucine aminopeptidase. *J. Histochem. Cytochem.*, 5: 264–278.

NAGABHUSHANAM, R. & SAROJINI, R., 1965, Note on the effects of experimental desiccation on three molluscs from Virginia. *Nassarius obsoletus*, *Branchidontes recurvus*, *Mya arenaria*. *Sci. Cult.*, 31(5): 257.

NEUBERG, C. & MANDL, I., 1950, Invertase *In*: *The Enzymes*, 1st ed. (J. B. Sumner & K Myrback, eds.) Academic Press, New York p 527–550.

NEWELL, R., 1965, The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. *Proc. zool. Soc. Lond.*, 144: 25–45.

OWEN, G., 1966, Digestion. *In*: *Physiology of Mollusca*, Vol. II. (K. Wilbur & C. M. Yonge, eds.) Academic Press, New York, p 53–96.

PAULSON, T. & SCHELTEMA, R., 1967, Selective feeding on algal cells by the veliger larvae of the gastropod *Nassarius obsoletus* (Say), *Amer. Zool.*, 7(4): 770–771.

PEARSE, A. G. E., 1960, *Histochemistry, Theoretical and Applied*. 2nd ed., Little, Brown & Co., Boston, 998 p.

PELSENEER, P., 1906, Mollusca. Part V. *In*: *A Treatise on Zoology*. (E. R. Lankester, ed.). Adam and Chas. Black, London, 355 p.

PIGMAN, W. W., 1941, Action of almond emulsin on the phenyl glycosides of synthetic sugars and on β-thiophenyl-D-glucoside. *J. Res. Natl. Bur. Stds.*, 26: 197–204.

PIGMAN, W. W., 1950, Cellulases, hemicellulases, and related enzymes. *In*: *The Enzymes*, 1st ed. (P. B. Sumner & K. Myrback, eds.) Academic Press, New York, p 725–739.

PRINTZ, D. W., 1962, Effects of parasitism on certain tissues of *Nassarius obsoletus*. *J. Elisha Mitchell sci. Soc.*, 78: 90.

PROSSER, C. L. & BROWN, F. A., 1961, *Comparative Animal Physiology*. 2nd ed. Saunders, Philadelphia, 688 p.

RANKIN, J. S., 1940, Studies on the trematode family Microphallidae Travassos, 1921. IV. The life cycle and ecology of *Gynaecotyla nassicola*. *Biol. Bull.*, 79: 439–451.

RICHARDSON, D., BERKOWITZ, S., & MOOG, F., 1955, The functional differentiation of the small intestine. V. The accumulation of non-specific esterase in the duodenum of chick embryos and hatched chicks. *J. exp. Zool.*, 130: 57–69.

ROBSON, G. C., 1922, On the connection between the style sac and intestine in Gastropoda and Lamellibranchia. *Proc. malac. Soc. Lond.*, 15: 41–46.

ROTHSTEIN, A., MEIER, R. & SCHAERF, T., 1953, The relationship of the cell surface to metabolism. IX. The digestion of phosphorylated compounds by enzymes located on the surface of the intestinal cell. *Am. J. Physiol.*, 173: 41–46.

RUTENBURG, A. M., RUTENBURG, S. H., MONIS, B., TEAGUE, R., & SELIGMAN, A. M., 1958, Histochemical demonstration of β-D-galactosidase in the rat. *J. Histochem. Cytochem.*, 6: 122–129.

SCHELTEMA, R. S., 1956, The effect of substrate on the length of planktonic existence in *Nassarius obsoletus*. *Biol. Bull.*, 111: 312.

SCHELTEMA, R. S., 1961, Metamorphosis of the veliger larvae of *Nassarius obsoletus* in response to bottom sediment. *Biol. Bull.*, 120: 92–109.

SCHELTEMA, R. S., 1962a, Pelagic larvae of New England intertidal gastropods, I. *Nassarius obsoletus* Say and *Nassarius vibex* Say. *Trans. Amer. microsc. Soc.*, 81: 1–11.

SCHELTEMA, R. S., 1962b, Environmental factors affecting length of pelagic development in the gastropod *Nassarius obsoletus*. *Amer. Zool.*, 2: 445.

SCHELTEMA, R. S., 1964, Feeding and growth in the mud snail, *Nassarius obsoletus*. *Chesapeake Sci.*, 5: 161–166.

SCHELTEMA, R. S., 1965, Relationship of salinity to larval survival and development in *Nassarius obsoletus*. *Biol. Bull.*, 129: 340–353

SELIGMAN, A. M., TSOU, K-C., RUTENBURG, S. H., & COHEN, R. B., 1954, Histochemical demonstration of β-D-glucuronidase with a synthetic substrate. *J. Histochem. Cytochem.*, 2: 209–229.

SELIGMAN, A. M. & NACHLAS, M. N., 1963, Lipase. *In*: *Methods of Enzymatic Analysis*. (H. Bergmeyer, ed.) Academic Press, New York, p 776–778.

SINDERMANN, C. J., 1960, Ecological studies of marine dermatitis-producing schistosome larvae in northern New England. *Ecology*, 41: 678–684.

SMITH, B. J., 1965, The secretions of the reproductive tract of the garden slug *Arion ater*. *Ann. N.Y. Acad. Sci.*, 118: 997–1014.

STEEDMAN, H. F., 1950, Alcian Blue 8GS. A new stain for mucin. *Quart. J. microsc. Sci.*, 91: 477–479.

STEPHENS, G. C., SANDEEN, M. I. & WEBB, H. M., 1953, A persistent tidal rhythm of activity in the mud snail *Nassa obsoleta*. *Anat. Rec.*, 117: 635.

STONE, B. A. & MORTON, J. E., 1959, The distribution of cellulases and related enzymes in the Mollusca. *Proc. malac. Soc. Lond.*, 33: 127–141.

STUNKARD, H. W., 1938a, The morphology and life cycle of the trematode *Himasthla quissetensis*. *Biol. Bull.*, 75: 145–164.

STUNKARD, H. W., 1938b, *Distomum lasium* Leidy, 1891 (Syn. *Cercariaeum lintoni* Miller and Northup, 1926), the larval stage of *Zoogonus rubellus* (Olsson, 1898). *Biol. Bull.*, 75: 308–334.

STUNKARD, H. W., 1961, *Cercaria dipterocerca* Miller and Northup, 1926 and *Stephanostomum dentatum* (Linton, 1900) Manter, 1931. *Biol. Bull.*, 120: 221–237.

STUNKARD, H. W. & HINCHLIFFE, H. B., 1952, The morphology and life history of *Microbilharzia variglandis* (Miller and Northup, 1926). *J. Parasitol.*, 38: 248–265.

SUMNER, A. T., 1965, The cytology and histochemistry of the digestive gland cells of *Helix*. *Quart. J. microsc. Soc.*, 106: 173–191.

SUMNER, A. T., 1966, The fine structure of digestive gland cells of *Helix*, *Succinea*, and *Testacella*. *J. Roy. microsc. Soc.*, 85: 181–192.

TALLAN, H., JONES, M. & FRUTON, J. S., 1952, On the proteolytic enzymes of animal tissues. X. Beef spleen Cathepsin C. *J. biol. Chem.*, 194: 793–805.

VEIBEL, S., 1950, β-Glucosidase. *In*: *The Enzymes*, 1st. ed. (P. B. Sumner & K. Myrback, eds.) Academic Press, New York, p 584–618.

WEICHSELBAUM, T. E. & SOMOGYI, M., 1941, A method for the determination of small amounts of ketone bodies. *J. biol. Chem.*, 140: 5–20.

WILBUR, K. & YONGE, C. M., 1964, *Physiology of Mollusca*, Vol. 1, Academic Press, New York, 473 p.

WU, S. K., 1965, Comparative functional studies of the digestive system of the muricid gastropods *Drupa ricina* and *Morula granulata*. *Malacologia*, 3: 211–234.

YONGE, C. M., 1930, The crystalline style of the Mollusca and a carnivorous habit cannot normally coexist. *Nature*, Lond., 125: 444–445.

YONGE, C. M., 1932, Notes on feeding and digestion in *Pterocera* and *Vermetus*, with a discussion on the occurrence of the crystalline style in the Gastropoda. *Sci. Rep. Gt. Barrier Reef Exped.*, 1: 259–281.

YONGE, C. M., 1954, Physiological anatomy of the alimentary canal in invertebrates. *Tabul. biol.*, Hague, 21(20): 1-24.

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RESUME

LA STRUCTURE ET LE FONCTIONNEMENT DE L'APPAREIL DIGESTIF DE LA NASSE, *NASSARIUS OBSOLETUS* (SAY)

S. C. Brown

La nasse des côtes américaines atlantiques, *Nassarius obsoletus* (Say) est un représentant des Gastropodes rachiglosses, typiquement carnivores. Dans la nature, cependant, *N. obsoletus* est un mangeur de détritus non sélectif, se nourrissant presque exclusivement par ingestion de sable et de boue. La présente étude a été entreprise pour clarifier le mécanisme du fonctionnement de l'appareil digestif de l'animal.

Des études anatomiques et histologiques indiquent que *Nassarius obsoletus* a toutes les modifications structurales associées à l'acquisition d'un mode de vie carnivore. Ces modifications comprennent: un proboscis allongé et extensible; une dentition radulaire rachidienne, un long siphon mobile et une osphradie bipectinée; un pharynx de Leiblein, une glande de Leiblein et des glandes salivaires bien développées; un estomac simple possédant un bouclier gastrique très reduit; pas d'airs de triage ciliées réellement efficaces; et des couches musculaires entourant le tube digestif fortement développées. En contraste avec ces caractéristiques clairement carnivores *N. obsoletus* possède un stylet cristallin mucoprotéique dans son estomac; c'est là un fait en relation avec une adaptation structurale à un régime herbivore. Des études histochimiques montrent que la glande digestive contient des enzymes capables de fractionner les esters et les glucuronides et donc de métaboliser quelques uns des principaux constituants des algues. Des

expériences de nutrition portant sur l'utilisation de matériel finement divisé en particules et sur la localisation de l'activité des phosphatases, montrent conjointement qu'il n'existe ni phagocytose, ni digestion intracellulaire. *In vitro* les analyses enzymatiques de tissus provenant des divers organes digestifs, révélent la présence d'estérase lipase, α-amylase, protéase et de plusieurs disaccharases. Des analyses du suc gastrique et du stylet cristallin révèlent, de façon similaire, la présence d'enzymes extracellulaires à l'intérieur de la lumière de l'estomac. Au cours d'examen des modes de nutrition et du comportement, il est apparu avec évidence que, physiologiquement, le stylet cristallin aide la digestion et qu'on doit par conséquent le considérer comme vraiment fonctionnel plutôt que comme un simple reste du cordon fécal muqueux.

Selon les données présentées, on en conclut que *Nassarius obsoletus*, bien que possédant structuralement toutes les caractéristiques d'un carnivore rachidien typique, est cependant capable de subsister presque entiérement avec un régime de détritus d'algues; qu'il possède les enzymes hydrolysantes nécessaires pour attaquer les principaux constituants des algues; que le début de l'hydrolyse est extra-cellulaire; que la phagocytose et la digestion intra-cellulaire n'ont pas lieu et que l'absorption des produits solubles de digestion a probablement lieu dans la glande digestive ou au niveau de l'épithelium qui sépare l'estomac de l'intestin.

A. L.

RESUMEN

ESTRUCTURA Y FUNCION DEL SISTEMA DIGESTIVO EN EL CARACOL DEL BARRO, *NASSARIUS OBSOLETUS* (SAY)

S. C. Brown

El caracol que habita los barros de la costa del Atlántico de Estados Unidos, *Nassarius obsoletus* (Say), pertenece al grupo de los gastrópodos raquiglosos tipicamente carnívoros; sin embargo, no selecciona su alimento y subsiste enteramente ingiriendo arena y barro. Este estudio aclara el mecanismo, y función, del sistema digestivo.

Estudios anatómicos e histológicos indican que *Nassarius obsoletus* tiene todas las modificaciones estructurales asociadas con una existencia carnívora. Estas modificaciones incluyen: proboscis alargada; rádula raquiglosa; sifón alargado y movible y un osfradio pectinado; válvula de Leiblein bien desarrollada y glándulas salivares; estómago simplificado con un escudo gástrico reducido; areas de seleccion ciliar no eficientes y tejido muscular bien desarrollado alrededor del canal alimenticio. En contraste con estas caracteristicas tan claramente carnívoras, posee en el estómago un estilete cristalino mucoproteico—asociado con la adaptación estructural para una dieta herbívora. Estudios histoquímicos indican que el intestino medio contiene enzimas capaces de desdoblar esterasa y glucoronidos, para metabolizar algunos de los constituyentes principales de las algas. Experimentos en nutrición, usando materiales finamente divididos y localización histoquímica de fosfatasa, indicaron que tanto la fagocitosis como la digestión intracelular no tienen lugar. Enzimas *in vitro* de tejidos de los diferentes órganos digestivos revelan la presencia de esterasa, lipasa, amilasa, proteasa y varios disacaridos. Anàlisis del fluido estomacal y estilete cristalino, ambos revelaron la presencia de enzimas hidrolíticas extracelularmente dentro del lúmen del estomago. La revisión de los hábitos alimenticios se presenta junto con la evidencia fisiológica de que el estilete cristalino ayuda en el proceso digestivo y es verdaderamente funcional, en vez de ser un remanente de la mucosa fecal.

En conclusion, aunque *Nassarius obsoletus* posee todas las condiciones tipicas de un carnívoro es, sin embargo, capaz de subsistir casi completamente de una dieta de detritos de algas; produce enzimas hidrolíticas para desdoblar los principales constituyentes de las algas; el desdoblamiento incial se produce extracelularmente; no hay caso de fagocitosis o digestión intracelular, y la absorción de los productos solubles de digestión ocurre probablemente en la glándula del intestino medio o en el revestimiento del estómago-intestino.

J. J. P.

АБСТРАКТ

СТРУКТУРА И ФУНКЦИЯ ПИЩЕВАРИТЕЛЬНОЙ СИСТЕМЫ ИЛОВОГО МОЛЛЮСКА *NASSARIUS OBSOLETUS* (SAY)

С. С. БРОУН

Иловая улитка американского атлантического побережья *Nassarius obsoletus* (Say) является представителем типичных хищных моллюсков из рахиглоссных Gastropoda. В природе, однако, *N. obsoletus* является безвыборочно-заглатывающим донные осадки: ил и песок. Настоящее исследование было предпринято для выяснения механизма работы пищеварительной системы этого моллюска.

Анатомическое и гистологическое исследование показывают, что *N. obsoletus* имеет все структурные модификации, связанные с предположительно хищным образом жизни, это: удлиненный вытягивающийся хобот, радула с рахиглоссными зубчиками, удлиненный подвижный сифон и двугребенчатый осфорадум; хорошо развитый клапан и железа Леблейна и слюнные железы; просто устроенный желудок с сильно редуцированным гастрическим щитком; отсутствие хорошо развитой ресничной области; хорошо развитые мускульные слои, окружающие пищеварительный тракт.

В противоположность этим признакам хищного образа питания, *N. obsoletus* обладает в желудке мукопротеиновым кристаллическом стебельком, т.е. органом, связанным с адаптацией к растительноядному типу питания. Гистохимическое изучение показывает, что железа средней кишки содержит энзимы, способные расщеплять эстэры, глюкороиды и таким образом усваивать основные компоненты водорослей. Опыты по питанию, когда употреблялись тонко растертые частицы пищи, а также гистохимическая локализация активности фосфатазы показали, что фагоцитоз и внутриклеточное переваривание не имеет места.

Энзимовый анализ гомогената тканей *in vitro*, взятых из различных пищеварительных органов, указывает на наличие эстеразы, α-амилазы, протеазы и некоторых дисахараз. Анализ желудочного сока и кристаллического стебелька сходным образом показал наличие экстрацеллюлярных гидролитических энзимов внутри желудка. Образ питания и поведения моллюсков, наряду с физиологическими данными, указывает, что кристаллический стебелек помогает процессу пищеварения и является истинно функциональным, а не остатком слизистого фекального тяжа.

Из полученных данных видно, что *Nassarius obsoletus*, хотя и обладает всеми структурными признаками типичного хищника из рахидоглоссных гастропод, тем не менее может существовать почти целиком на водорослевом детрите. Он обладает гидролитическими энзимами, необходимыми для расщепления основных компонентов водорослей. Первичное расщепление происходит внеклеточно. Фагоцитоз и внутриклеточное переваривание не наблюдаются. Всасывание растворенных пищевых веществ может встречаться наиболее вероятно в средней кишке или в эпителии, выстилающем внутренность желудка.